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**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371**

U.S. APPLICATION NO.

09/367496INTERNATIONAL APPLICATION NO.
PCT/FR98/00328INTERNATIONAL FILING DATE
19 FEBRUARY 1998PRIORITY DATE CLAIMED
19 FEBRUARY 1997

TITLE OF INVENTION: USE OF ULIP PROTEINS IN THE DIAGNOSIS AND THERAPY OF CANCER...

APPLICANT(S) FOR DO/EO/US: AGUERA, Michele et al.

Applicant herewith submits to the US Designated/Elected Office (DO/EO/US) the following items and other information:

- ☒ 1. This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
- ☐ 2. This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 USC 371.
- ☒ 3. This express request to begin national examination procedures (35 USC 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 USC 371(b) and PCT Art. 22 and 39(1).
- ☒ 4. A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
- ☒ 5. A **copy** of the International Application as filed (35 U.S.C. 371 (c)(2))
- ☐ a. is transmitted herewith (required only if not transmitted by the International Bureau).
- ☒ b. has been transmitted by the International Bureau.
- ☐ c. is not required, as the application was filed in the United States Receiving Office (RO/US).
- ☐ 6. A **translation** of the International Application into English (35 U.S.C. 371(c)(2)).
- ☒ 7. Amendments to the claims of the International Appln. under PCT Article 19 (35 USC 371 (c)(3))
- ☐ a. are transmitted herewith (required only if not transmitted by the International Bureau).
- ☐ b. have been transmitted by the International Bureau.
- ☐ c. have not been made; however, the time limit for making such amendments had NOT expired.
- ☒ d. have not been made and will not be made.
- ☐ 8. A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
- ☐ 9. An **oath** or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
- ☐ 10. A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

- ☐ 11. An **Information Disclosure Statement** under 37 C.F.R. 1.97 and 1.98.
- ☐ 12. An **Assignment** document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
- ☐ 13. A **First preliminary amendment**.
- ☐ A Second or subsequent preliminary amendment.
- ☐ 14. A substitute specification.
- ☐ 15. A change of power of attorney and/or address letter.
- ☐ 16. Other items or information:
- ☐ **Small Entity Statement**
- ☐
- ☐ A copy of the Notification of Missing Requirements under 35 U.S.C. 371.
- ☐ In the event that a petition for extension of time is required to be submitted herewith, and in the event that a separate petition does not accompany this response, applicant hereby petitions under 37 CFR 1.136(a) for an extension of time of as many months as are required to render this submission timely. Any fee is authorized in 17(c).

Date: 17 August 1999

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17 AUG 1999

INTERNATIONAL APPLICATION NO. PCT/FR98/00328		INTERNATIONAL FILING DATE 19 FEBRUARY 1998		PRIORITY DATE CLAIMED 19 FEBRUARY 1997	
<input checked="" type="checkbox"/> 17. The following fees are submitted: <input checked="" type="checkbox"/> Basic National Fee (37 CFR 1.492 (a) (1)-(5):					
<input checked="" type="checkbox"/> Search Report has been prepared by the EPO or JPO \$ 840 <input type="checkbox"/> International preliminary examination fee paid to USPTO \$ 670 <input type="checkbox"/> No Int'l Prelim. Ex. fee paid to USPTO but Int'l Search fee paid to USPTO \$ 760 <input type="checkbox"/> Neither Int'l Prelim. Exam. fee nor Int'l Search fee paid to USPTO \$ 970 <input type="checkbox"/> Int'l Prelim. Ex. fee paid to USPTO & all claims satisfied PCT Art. 33(1)-(4) \$ 96					
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$ 840.00	
<input type="checkbox"/> Surcharge of \$130 for furnishing the oath or declaration later than from the earliest claimed priority date (37 CFR 1.492(e)).				<input type="checkbox"/> 20 mos. <input type="checkbox"/> 30 mos. +	\$
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	- 20 =		X \$18 =	\$	
Independent Claims	- 03 =		X \$78 =	\$	
<input type="checkbox"/> Multiple Dependent Claim(s) (if applicable)			+ \$260 =	\$	
TOTAL OF ABOVE CALCULATIONS =				\$ 840.00	
<input type="checkbox"/> Reduction of 1/2 for filing by small entity, if applicable. Small Entity Statement must also be filed.				\$	
SUBTOTAL =				\$ 840.00	
<input type="checkbox"/> Processing fee of \$130 for furnishing the English translation later than from the earliest claimed priority date (37 CFR 1.492(f)).				<input type="checkbox"/> 20 mos. <input type="checkbox"/> 30 mos. +	\$
TOTAL NATIONAL FEE =				\$ 840.00	
<input type="checkbox"/> Fee for recording the enclosed assignment, accompanied by a cover sheet - \$40 per property				\$	
TOTAL FEES ENCLOSED =				\$ 840.00	
Amount to be				Refunded	\$
				Charged	\$
<input checked="" type="checkbox"/> a. A check in the amount of \$ 840.00 to cover the above fees is enclosed. <input type="checkbox"/> b. Please charge my Deposit Account No. 12-0555 in the amount of \$ to cover the above fees. <input checked="" type="checkbox"/> c. The Commissioner is hereby authorized to charge any additional fees required or credit overpayment to Deposit Account No. 12-0555.					
Note: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
Address correspondence to: THOMAS P. SARRO			SIGNATURE: <u>Douglas E. Jackson</u> NAME: Douglas E. Jackson REG. NO.: 28518 PHONE NO.: 703-739-4900 Date: 17 August 1999		
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PCT/FR98/00328

Use of ULIP proteins in the diagnosis and therapy of
cancers and paraneoplastic neurological syndromes

The invention relates to the use of proteins designated ULIP/POP in the diagnosis and therapy of
5 cancers and paraneoplastic neurological syndromes.

Paraneoplastic neurological syndromes (PNS) occur in the instance of a cancer, often before its discovery, and are not connected either to the tumour proliferation itself (direct invasion, metastases) or
10 to the therapy. Their frequency is globally estimated at approximately 1% of cancers. Several clinical pictures have been individualized for a long time (encephalomyelitis, Denny-Brown's sensitive neuropathy, cerebellar atrophy, limbic encephalitis,
15 opsoclonus,...) corresponding in fact to the either elective or preferential attack of certain groups of neurons. The frequency of inflammatory cells in the neighbourhood of the lesions for numerous years brought to mind the possibility of an auto-immune or viral
20 process. The more recent demonstration of auto-antibodies in the serum and the cerebrospinal fluid (CSF) of patients suffering from PNS, specific to the type of tumour and the type of neurons which degenerate, has revived the hypothesis of participation
25 of auto-immunity in the genesis of this pathology (Graus et al., 1985; Greenlee et al., 1983).

Apart from the presence of a high titre of these antibodies in the blood and the CSF of patients, there are several arguments suggesting that PNS depend
30 on auto-immune mechanisms. Thus the antigens recognized in the central nervous system are also present in the tumours of patients (Anderson et al., 1987). At the level of the tumour tissue, antibodies specifically directed against these antigens as well as B and T
35 lymphocytes are found (Hetzl et al., 1990).

These data suggest that the auto-immune process could be triggered by the expression of tumour antigens. A cross-immunity process could provoke the lesions of the central nervous system. Other arguments

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additionally indicate that the cerebral lesions result from the auto-immune response. Thus, in the brain of the patients, the titre of specific antibodies is higher than that of the serum and the CSF (Dalmau et al., 1991). In addition, in the case of encephalomyelitis associated with anti-Hu antibodies, there is an intense lymphocytic reaction, made up of B and T cells, situated in proximity to neurons in the process of destruction (Dalmau et al., 1991; Graus et al., 1990).

Several types of auto-antibodies allowing precise syndromic groupings as a function of immunological, neurological and carcinogenic criteria have been described.

Thus, anti-Yo antibodies are found in the serum and the CSF of women having paraneoplastic cerebellar atrophy and a gynaecological cancer (ovary, breast or uterus) (Greenlee et al., 1983; Jaekle et al., 1985).

These antibodies recognize two cytoplasmic proteins of 34 and 62 kDa specific to Purkinje cells of the cerebellum.

The anti-Ri antibodies are found in the serum and the CSF of patients (principally of women) having opso-myoclonus, cerebellar syndrome and breast cancer. These antibodies recognize two proteins of 50 and 80 kDa specific to neurons of the central nervous system (Luque et al., 1991).

Anti-Hu antibodies are most frequently found in the course of PNS. They are found in the serum and the CSF of patients having Denny-Brown's syndrome or encephalomyeloneuritis and small-cell lung cancer (Graus et al., 1985; Dalmau et al., 1992). These auto-antibodies recognize several proteins of 37 to 45 kDa expressed specifically by all the neurons of the nervous system.

Another type of auto-antibody has recently been identified in patients having PNS: anti-CV2 antibodies (Antoine et al., 1993; Honnorat et al., 1996). The latter are atypical, in the sense that the antigenic

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target recognized in adulthood is essentially non-neuronal, although the post-mortem analysis of the brain of four patients allows neuronal loss, gliosis and an inflammatory process characteristic of PNS to be objectivized.

The originality of the discovery of these auto-antibodies resides, on the one hand, in their demonstration. The latter escaped all the usual investigations which consisted in revealing the antigens recognized by immunohistochemistry on post-mortem brain. The antigen recognized is indeed soluble and disappears from post-mortem brain under the majority of fixation conditions. Only fixation of human post-mortem tissue by immersion in paraformaldehyde or in situ by perfusion of paraformaldehyde in animals has allowed the presence of these antibodies in the CSF or the serum of patients suffering from PNS to be revealed (Antoine et al., 1993; Honnorat et al., 1996).

The anti-CV2 auto-antibodies present in the sera of patients suffering from paraneoplastic neurological syndrome (PNS) have been defined by their capacity to recognize, by indirect immunohistochemistry, a cytoplasmic antigen expressed specifically, in adult rat brain, by a subpopulation of oligodendrocytes of the brain stem, the medulla and the cerebellum.

The originality of these auto-antibodies resides, on the other hand, in their diagnostic interest. Their presence in the serum or the CSF of patients is of diagnostic value because it allows the paraneoplastic origin of a neurological syndrome to be specified. The discovery of these antibodies, when it precedes that of cancer, directs the search to that and allows its discovery. Such was the case for six patients out of 19 having anti-CV2 antibodies. The clinical disorders were different according to the patients, certain of them having a picture of limbic encephalitis, others encephalomyeloneuritis and others Lambert-Eaton syndrome. Nevertheless, in more than 60%

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of the cases, the cerebellar syndrome was predominant. The most frequently associated tumour was small-cell lung cancer (60% of the cases).

Experiments on the brains of newborn rats showed that these anti-CV2 antibodies reacted with a protein of 66 kDa (Honnorat et al., 1996).

In the adult brain, this antigen is situated in a subpopulation of oligodendrocytes or in cells which retain differentiation capacities in the adult brain (olfactory bulb, dentate gyrus). The recognized antigen could play a role in neuronal survival, via Neuron/Oligodendrocyte interactions, as the loss of neurons observed in the post-mortem brain of patients suffering from PNS suggests.

Its very limited expression in adulthood contrasts with a very strong and transitory expression in the central and peripheral nervous system in development, suggesting the probable role of this antigen in the development of the nervous system.

The Applicant has characterized the target antigen of anti-CV2 antibodies, which corresponds to a protein designated below by "POP-66" for "paraneoplastic oligodendrocyte protein 66 kDa".

Surprisingly, it has been discovered that the POP-66 protein belongs to the so-called ULIP family of proteins (for Unc-33-like phosphoprotein), involved in the control of neuronal development and axonal transport (T. Byk et al., 1996), and also studied in the form of CRMP proteins (Goshima et al., 1995, Wang et al., 1996), TOAD-64 (Minturn et al., 1995) and DRPs (Hamajima et al., 1996). More precisely, POP-66 has been identified as in fact being the human form of ULIP-4.

All of the data described below emphasize the complexity of this family of proteins, the existence of a very wide expression spectrum of members of this family in the brain in the course of ontogenesis, but a very limited spectrum in adults, as well as the

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specificity of the anti-CV2 antibodies for a member of this ULIP protein family, which is in fact POP-66.

Thus, the Applicant has shown that the protein recognized by the anti-CV2 antibodies of patients suffering from PNS is POP-66/ULIP-4 and has established the involvement of the ULIP proteins in paraneoplastic neurological syndromes and associated cancers. In addition to their role in cancers associated with PNS, the Applicant has likewise discovered that the proteins of the ULIP family could play a role in any other form of cancer not associated with PNS. More particularly, the ULIP proteins could especially be involved in cancers of tissues having a common embryonic origin with the central nervous system.

The present invention therefore relates to a purified ULIP polypeptide, derivative or polypeptide fragment of the said purified polypeptide, comprising an amino acid sequence selected from SEQ ID No. 2, No. 4, No. 6 and No. 8.

Preferentially, the present invention relates to a purified polypeptide, derivative, or biologically active polypeptide fragment of the said purified polypeptide, comprising the amino acid sequence SEQ ID No. 8, the said polypeptide being designated by "POP-66/ULIP-4".

A fragment of the polypeptide of sequence SEQ ID No. 8 of interest is, in particular, the antigenic fragment PARASCPGKIS (amino acids No. 517 to No. 527).

In the description of the invention, the following definitions are used:

- derivative: any variant polypeptide of the polypeptide of sequence SEQ No. 2, No. 4, No. 6 or No. 8 or any other molecule resulting from a modification of genetic and/or chemical nature of the sequence SEQ ID No. 2, No. 4, No. 6 or No. 8, that is to say obtained by mutation, deletion, addition, substitution and/or chemical modification of a single or of a limited number of amino acids, as well as any

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isoform sequence, that is to say a sequence identical to the sequence SEQ ID No. 2, No. 4, No. 6 or No. 8, to one of its fragments or modified sequences, containing one or more amino acids in the D enantiomer form, the
5 said modified or isoform variant sequences having conserved at least one of the properties making them biologically active.

- biologically active: having properties of induction and/or control of neuronal development and/or
10 antigenic properties.

The invention likewise relates to an isolated nucleic acid sequence selected from SEQ ID No. 1, No. 3, No. 5 and No. 7 or a sequence derived from the sequences SEQ ID No. 1, No. 3, No. 5 and No. 7 on
15 account of the degeneracy of the genetic code, or on account of mutation, of deletion or of insertion of at least one nucleotide, the said derived sequences having a biological activity virtually identical to that of the peptide encoded by the sequences SEQ ID No. 1,
20 No. 3, No. 5 and No. 7.

The various nucleotide sequences of the invention can be of artificial or non-artificial origin. They can be DNA or RNA sequences, obtained by screening of banks of sequences by means of probes
25 elaborated on the basis of sequences selected from SEQ ID No. 2, No. 4, No. 6 and No. 8. Such banks can be prepared by conventional techniques of molecular biology known to the person skilled in the art.

The nucleotide sequences according to the
30 invention can likewise be prepared by chemical synthesis, or alternatively by mixed methods including the chemical or enzymatic modification of sequences obtained by screening of banks.

These nucleotide sequences allow the production
35 of nucleotide probes capable of hybridizing strongly and specifically with a nucleic acid sequence of a genomic DNA or of a messenger RNA coding for a peptide according to the invention or a biologically active fragment of this. The appropriate hybridization

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conditions correspond to the conditions of temperature and of ionic strength usually used by the person skilled in the art (Sambrook et al., 1989), preferably to temperature conditions of between (T_m minus 5°C) and 5 T_m minus 30°C) and more preferably to temperature conditions of between (T_m minus 5°C) and (T_m minus 10°C) (great stringency), T_m being the theoretical melting point, defined as being the temperature at which 50% of the paired strands separate. Such probes are likewise 10 part of the invention. They can be used as a diagnostic tool in vitro for the detection, by hybridization experiments, of specific transcripts of polypeptides of the invention in biological samples or for the demonstration of aberrant syntheses or genetic 15 anomalies resulting from polymorphism, mutations or bad splicing.

The probes of the invention contain at least 10 nucleotides, and at most contain the whole of a nucleotide sequence selected from SEQ ID No. 1, No. 3, 20 No. 5 and No. 7 or of their complementary strand.

The in vitro diagnostic methods in which these nucleotide probes are employed for the detection of aberrant syntheses or genetic anomalies, such as the loss of heterozygosity and genetic rearrangement, at 25 the level of nucleic sequences coding for a ULIP polypeptide according to the invention or a biologically active fragment are included in the present invention. Such a method type comprises:

- the contacting of a nucleotide probe of the 30 invention with a biological sample under conditions allowing the formation of a hybridization complex between the said probe and the abovementioned nucleotide sequence, optimally after a previous amplification step of the abovementioned nucleotide 35 sequence;

- the detection of the hybridization complex optimally formed;

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- optimally the sequencing of the nucleotide sequence forming the hybridization complex with the probe of the invention.

The cDNA probes of the invention can additionally be advantageously used for the detection of chromosomal anomalies.

The nucleotide sequences according to the invention are likewise useful for the production and use of sense and/or antisense oligonucleotide primers for sequencing reactions or specific amplification reactions according to the so-called PCR technique (polymerization chain reaction) or any other variant of this.

The nucleotide sequences according to the invention additionally have uses in the therapeutic field, for the production of antisense sequences capable of hybridizing specifically with a nucleic acid sequence, including a messenger RNA, which can be used in gene therapy. The invention thus relates to antisense sequences capable of inhibiting, at least partially, the production of a polypeptide according to the invention, such as defined above.

They are more particularly useful in the treatment of disorders of the central and peripheral nervous system and of vision, especially in the treatment of paraneoplastic neurological syndromes, as well as in anti-cancer treatment, especially of tumours associated with paraneoplastic neurological syndromes.

The nucleotide sequences according to the invention can additionally be used for the production of recombinant ULIP proteins according to the invention.

These proteins can be produced from nucleotide sequences defined above, according to techniques of production of recombinant products known to the person skilled in the art. In this case, the nucleotide sequence used is placed under the control of signals allowing its expression in a cell host.

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An efficacious system of production of a recombinant protein necessitates having a vector, for example of plasmid or viral origin, and a compatible host cell.

5 The cell host can be selected from prokaryotic systems, such as bacteria, or eukaryotic systems, such as, for example, yeasts, insect cells, CHO (Chinese hamster ovary) cells or any other system advantageously available. A preferred cell host for the expression of
10 proteins of the invention is formed by the bacterium *E. coli*.

 The vector must contain a promoter, translation initiation and termination signals, as well as the appropriate regions of transcription regulation. It
15 must be able to be maintained stably in the cell and can possibly possess special signals specifying the secretion of the translated protein.

 These different control signals are selected as a function of the cell host used. To this end, the
20 nucleotide sequences according to the invention can be inserted in autonomous replication vectors within the selected host, or integrative vectors of the selected host. Such vectors will be prepared according to methods currently used by the person skilled in the
25 art, and the resulting clones can be introduced into an appropriate host by standard methods, such as, for example, electroporation.

 The invention is additionally directed at the host cells transfected by these above vectors. These
30 cells can be obtained by the introduction into host cells of a nucleotide sequence inserted into a vector such as defined above, then the culturing of the said cells under conditions allowing the replication and/or expression of the transfected nucleotide sequence.

35 These cells can be used in a method of production of a recombinant polypeptide according to the invention or any fragment or biologically active derivative of this.

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The method of production of a polypeptide of the invention in recombinant form is itself included in the present invention, and is characterized in that the transfected cells are cultured under conditions
5 allowing the expression of a recombinant polypeptide according to the invention or of any fragment or biologically active derivative of this, and in that the said recombinant polypeptide is recovered.

The purification processes used are known to
10 the person skilled in the art. The recombinant polypeptide can be purified from lysates and cell extracts, from the supernatant of the culture medium, by methods used separately or in combination, such as fractionation, chromatographic methods, immunoaffinity
15 techniques with the aid of specific mono- or polyclonal antibodies, etc:

One variant consists in producing a recombinant polypeptide fused to a "carrier" protein (chimeric protein). The advantage of this system is that it
20 allows a stabilization and a decrease in the proteolysis of the recombinant product, an increase in the solubility in the course of the *in vitro* renaturation and/or a simplification of the purification when the fusion component has an affinity
25 for a specific ligand.

The exploitation of ULIP proteins, and in particular POP-66/ULIP-4, as well as antibodies directed against these proteins, is promising in various fields.

30 Thus, the detection of the anti-CV2 auto-antibody by immunofluorescence on fixed animal brain is currently used as a diagnostic test.

The production of POP-66/ULIP-4 recombinant protein according to the invention allows the
35 production of a rapid and reliable test (of Elisa or Western Blot type) for detecting anti-CV2 antibodies.

Such tests already exist for anti-Hu, anti-Yo and anti-Ri antibodies. The test for detecting anti-CV2 in the serum of patients could be prescribed in the

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case of suspicion of paraneoplastic neurological syndrome and consequently could include anti-CV2 antibodies at the same titre as the other antibodies identified in the PNS such as mentioned above.

5 The invention is therefore likewise directed at a method for the diagnosis of paraneoplastic neurological syndromes and/or for the early diagnosis of the formation of tumours of cancerous origin, characterized in that auto-antibodies directed against
10 a POP-66/ULIP-4 protein are demonstrated in a blood sample taken from an individual by

- the contacting of a blood sample taken from an individual with a purified polypeptide (POP-66), derivative or biologically active polypeptide fragment
15 of POP-66/ULIP-4 optionally attached to a support under conditions allowing the formation of specific immunological complexes between the said polypeptide and the auto-antibodies optionally present in the serum sample, and

20 - the detection of the specific immunological complexes optionally formed.

The invention likewise relates to a kit for the diagnosis of paraneoplastic neurological syndromes and for the early diagnosis of the formation of tumours
25 from a biological sample, comprising:

- at least one purified POP-66/ULIP-4 polypeptide, derivative or biologically active polypeptide fragment of POP-66/ULIP-4, optionally attached to a support,

30 - means of visualization of the formation of specific antigen/antibody complexes between an anti-POP-66 auto-antibody and the said purified POP-66 polypeptide, derivative or polypeptide fragment and/or means of quantification of these complexes.

35 The invention likewise relates to mono- or polyclonal antibodies or their fragments, chimeric or immunoconjugated antibodies obtained from a purified ULIP polypeptide comprising an amino acid sequence selected from SEQ ID No. 2, No. 4, No. 6 and No. 8,

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derivative or biologically active polypeptide fragment of ULIP and their use for the purification or the detection of a ULIP protein in a biological sample.

Polyclonal antibodies can be obtained from the serum of an animal immunized against the protein, produced, for example, by genetic recombination according to the method described above, according to the usual working methods.

The monoclonal antibodies can be obtained according to the conventional method of hybridoma culture described by Köhler and Milstein.

The antibodies can be chimeric antibodies, humanized antibodies, Fab and F(ab')₂ fragments. They can likewise be present in the form of immunoconjugates or labelled antibodies.

The invention likewise relates to the use of antibodies directed against a protein of the ULIP family for the demonstration of a ULIP protein in neoplasms, and paraneoplastic neurological syndromes for diagnostic purposes.

Preferentially, the invention relates to the use of monoclonal antibodies obtained from polyclonal anti-CV2 serum of patients by immortalization of lymphocytes, according to the usual techniques known to the person skilled in the art.

Thus, the antibodies directed against a protein of the ULIP family are useful for detecting abnormal expression of ULIP protein in patients having neurological syndromes, in whom cancer has not been diagnosed by the conventional methods. This abnormal expression of ULIP protein will be able to be correlated with the existence of a cancer which had not been spotted. Thus, the antibodies directed against a ULIP protein, especially against POP-66/ULIP-4, are useful for the early diagnosis of cancer.

The invention likewise relates to a method of determination of an allelic variability, a mutation, a deletion, an insertion, a loss of heterozygosity or a genetic anomaly of the POP-66/ULIP-4 gene, situated on

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chromosome 10 in the 26q region and which can be involved in pathologies, characterized in that it employs at least one nucleotide sequence SEQ ID No. 7. Amongst the methods of determination of an allelic variability, a mutation, a deletion, an insertion, a loss of heterozygosity or a genetic anomaly of the POP-66/ULIP-4 gene, a method comprising at least one PCR amplification step of the nucleic sequence of POP-66/ULIP-4 capable of having a polymorphism, a mutation, a deletion or an insertion with the aid of pairs of primers of nucleotide sequences, a step in the course of which amplified products are treated with the aid of appropriate restriction enzymes and a step in the course of which at least one of the products of the enzymatic reaction is detected or determined is preferred.

Advantageously, it is possible to search for the mutations associated with the said chromosome 10 in relation to cancer, especially peripheral cancerous tumours and primitive cerebral tumours of glial origin, for example.

The invention likewise relates to a pharmaceutical composition comprising at least one purified protein of the ULIP family, polypeptide fragment or biologically active derivative of this, a nucleotide sequence or nucleotide sequence fragment coding for the said protein, an antisense sequence capable of hybridizing specifically with a nucleotide sequence coding for the said protein, or an antibody directed against the said protein, combined with a pharmaceutically acceptable vehicle.

The invention preferentially comprises pharmaceutical compositions comprising as active principle a purified POP-66 polypeptide, derivative or polypeptide fragment of POP-66, preferentially in soluble form, combined with a pharmaceutically acceptable vehicle.

Such compositions offer a new approach to treating disorders of the central and peripheral

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nervous system and of vision, and especially
paraneoplastic neurological syndromes. In addition,
they are useful for treating neurological disorders
connected with a neuronal loss and/or an
5 underexpression of ULIP proteins in the nervous system.

Thus, POP-66/ULIP-4 is also of interest in
neurodegenerative pathologies such as multisystemic
atrophies which are conditions similar to those of PNS
and for which an anomaly of an oligodendrocytic
10 subpopulation has been detected (Papp et al., 1992).

The compositions according to the invention are
additionally useful in anticancer therapy.

The antibodies directed against one or more
ULIP proteins can be combined with antineoplastic
15 agents, thus allowing the targeting of medicaments
towards the tumour cells.

They can additionally be combined with a
hydrophilic chemical group chosen in such a way so as
to cross or not to cross the blood-brain barrier,
20 according to the type of tumour.

The ULIP proteins and in particular POP-66 as
well as the nucleotide sequences coding for the said
proteins and the antisense sequences or
oligonucleotides can be useful in the therapy of any
25 type of cancer in which a gene coding for a ULIP
protein is involved. Amongst examples of cancers, it is
possible to mention peripheral tumours, such as small-
cell lung cancer, thymoma, cancer of the breast and of
the ovary, as well as cerebral tumours, preferably
30 primitive cerebral tumours of glial origin. The
expression of POP-66 in the non-proliferative cells of
normal brain, its absence in normal tissues such as
lung or thymus, for example, its differential
reexpression during tumorigenesis of these tissues and
35 the modulation of its expression in a tumour line in
the course of differentiation suggest in this respect
that POP-66 could be a tumour suppressor gene.

Preferentially, the pharmaceutical compositions
according to the invention can be administered by the

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systemic route, preferably by the intravenous route, by the intramuscular route, intradermally or by the oral route.

5 Their modes of administration, dosages and optimal pharmaceutical forms can be determined according to the criteria generally taken into account in the establishment of a therapeutic treatment adapted to a patient, such as, for example, the age or the body weight of the patient, the seriousness of his/her
10 general condition, the tolerance to the treatment and the secondary effects noted, etc.

The invention likewise comprises the use of a purified protein of the ULIP family, polypeptide fragment or biologically active derivative of this, a
15 nucleotide sequence or nucleotide sequence fragment coding for the said protein, an antisense sequence capable of hybridizing specifically with a nucleotide sequence coding for the said protein, or an antibody directed against the said protein, combined with a
20 pharmaceutically acceptable vehicle, for the production of a medicament intended for treating neurodegenerative illnesses and neoplasms.

The invention finally relates to a method of treatment of neurodegenerative illnesses and neoplasms,
25 comprising the administration to a subject requiring such a treatment of a therapeutically efficacious quantity of a purified protein of the ULIP family, polypeptide fragment or biologically active derivative of this, a nucleotide sequence or nucleotide sequence
30 fragment coding for the said protein, an antisense sequence capable of hybridizing specifically with a nucleotide sequence coding for the said protein, or an antibody directed against the said protein, combined with a pharmaceutically acceptable vehicle.

35 The examples and the figures whose legends are presented below are given by way of illustration.

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LEGEND TO THE FIGURES

- Figure 1 represents a two-dimensional electrophoresis profile obtained from brain protein
5 extracts of newborn rats enriched in POP-66.

A: silver staining of all of the proteins.

B: immunoblot with the anti-CV2 serum of patients.

The arrows indicate the spots corresponding to
10 POP-66, revealed with anti-CV2 antibodies.

- Figure 2 represents a two-dimensional electrophoresis profile obtained from protein extracts of brains of newborn rats.

Immunoblot with A- antipeptide antibody 3 and
15 B- anti-CV2 antibody.

- Figure 3 represents a one-dimensional electrophoresis obtained from protein extracts of brains of newborn rats.

Immunoblot with a: preimmune serum for peptide 3

20 Immunoblot with b: anti-peptide serum 3

Immunoblot with c: anti-peptide serum 4

Immunoblot with d: preimmune serum for peptide 4.

- Figure 4 represents an immunohistochemical labelling of sections of brains of adult rats with

25 A: anti-CV2 serum of a patient suffering from PNS

B: rabbit serum with anti-peptide 3 antibodies

C: rabbit serum with anti-peptide 4 antibodies.

- Figure 5 represents a histological labelling
30 of sections of young rat cerebellum 8 days post-natally.

A: Staining with toluidine blue; ge = external granular layer; m = molecular layer (x400).

B: Immunolabelling after incorporation of BrdU
35 (bromodeoxyuridine). The cells which have incorporated BrdU are virtually all situated in the most external zone of the external granular layer (ge). Some positive cells are situated in the internal granular layer (x400).

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C: Indirect immunoperoxidase with a patient serum containing an anti-CV2 antibody (x400). The immunoreactivity is concentrated in the internal part of the external granular layer (future molecular layer (m)). Some cells are immunoreactive in the internal granular layer. The Purkinje cells (p) are negative as well as the cells of the external part of the external granular layer (ge).

D: Indirect immunoperoxidase with a patient serum containing an anti-CV2 antibody (x1000). Above all, the immunolabelling is concentrated in the internal part of the external granular layer (future molecular layer (m)). A reactive cell is noted in the internal granular layer (gi) (arrow).

- Figure 6 represents immunohistochemical labelling of sections of post-mortem hippocampus (HPS staining).

A: brain of control patient,

B: brain of patient having limbic encephalitis, and circulating anti-CV2 antibody. It is possible to note the disappearance of the granular cells.

- Figure 7 represents a two-dimensional electrophoresis profile with the control ULIP-2 protein (A) and the ULIP-4 protein (B).

Figure 7C represents the migration profile model of the proteins ULIP-1, 2, 3 and 4 as a reference.

The proteins are revealed:

a) by autoradiography to locate the proteins translated *in vitro* (translation);

b) by immunoblotting with the anti-CV2 serum.

- Figure 8 represents a migration profile of the mRNA of C-22/ULIP-3 (8A) and TOAD-64/ULIP-2 (8B) amplified by RT-PCR expressed in different cell types:

lanes 1-3:	small-cell lung tumour
lane 2:	small-cell lung tumour with anti-CV2 serum
lane 4:	control cDNA.

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lane 5: medulloblastoma treated by
HTLV1 infection

lanes 6-7: medulloblastoma

lane 8: C6 line of glial cells in mice

5 lane 9: control

lane 10: nothing

lane 11: kb scale.

10 The black arrows correspond to POP-66; the
white arrows correspond to the molecular weight
standard.

- Figure 9 represents the nucleotide sequence
of ULIP-2 in mice (SEQ ID No. 1), as well as the
inferred amino acid sequence (SEQ ID No. 2).

15 - Figure 10 represents the nucleotide sequence
of ULIP-3 in mice (SEQ ID No. 3), as well as the
inferred amino acid sequence (SEQ ID No. 4).

- Figure 11 represents the nucleotide sequence
of ULIP-4 in mice (SEQ ID No. 5), as well as the
inferred amino acid sequence (SEQ ID No. 6).

20 - Figure 12 represents the nucleotide sequence
of ULIP-4 in man (SEQ ID No. 7), as well as the
inferred amino acid sequence (SEQ ID No. 8).

25 An erroneous stop codon in the human ULIP-4
sequence (asterisk) arises from a fault of the reverse
transcriptase in the production of the bank. By
comparing with ULIP-4 of rats and of mice, it is almost
certain that the TAG sequence coding for a stop is in
fact an AAG codon, coding for a lysine as in rats and
mice. In addition, the region around this amino acid is
30 entirely conserved in the three species.

The amino acid sequence has been completed in
SEQ ID No. 8 by 15 C-terminal amino acids (No. 554 to
No. 568). This C-terminal region which is missing in
Figure 12 is very well conserved between rat and mice
35 ULIP-4 as well as between the different ULIPs.

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EXAMPLE 1:**Purification of POP-66 and sequencing**

5 The purification of POP-66 is carried out according to the material and the methods described in the article of Honnorat et al., 1996, incorporated by reference, starting from serum of patients suffering from PNS.

10 To identify the protein POP-66, a purification strategy was chosen which allows a partial sequencing to be obtained. The screening of an expression bank of brain cDNA or the immunoaffinity purification of the protein were excluded because of the limited quantities of sera linked to the death of the patients. It was possible to develop a method of biochemical purification starting from brains of newborn rats on account of the anti-CV2 human sera, which allowed each purification step to be monitored.

20 The tissues, preserved at -70°C before use, were treated with a solution containing 0.2 M DTT (dithiothreitol) (Sigma) 2% Ampholine 3-10 (Pharmacia), 2% Triton X-100 (Merck) and placed at 2-4°C. Immediately before use, solid urea (Pharmacia) was added to obtain an 8M solution.

25 The POP-66 protein is soluble, at least in part, and precipitates entirely at a concentration of 40% ammonium sulphate.

30 Centrifugation at 100,000 (times) g and ammonium sulphate precipitation (eliminating the proteins precipitating below 20% and above 40% ammonium sulphate) allows protein extracts enriched in POP-66 to be obtained. The proteins of this extract are then separated, after dialysis, by isofocussing on agarose gel (Peltre et al., 1982).

35 After transfer to a membrane, the anti-CV2 antibodies recognize several bands of isoelectric points of between 5.85 and 6.55. All of these bands correspond to the POP-66 protein recognized by the anti-CV2 antibodies. This spectrum suggests the

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possibility of transcriptional modifications (phosphorylations and/or glycosylations) of the protein.

The zone of proteins of pI between 5.85 and 6.55 from the agarose gel is used for a new electrophoretic migration in denaturing medium on polyacrylamide gel previously equilibrated with an equilibration solution (0.05 mol/l Tris/HCl, pH 6.8, 6M urea, 30% glycerol, 1% weight/volume SDS for 2 x 10 minutes) to which is added DTT (0.25% weight/volume) and bromophenol blue.

Two methods of detection are used:

- *silver staining*. Immediately after the end of the migration, the gel is immersed in a fixing solution (40% ethanol, 10% acetic acid) for 30 minutes; it is then placed in an incubation solution (30% ethanol, 7% weight/volume of sodium acetate, 0.1% glutaraldehyde, 0.2% weight/volume of sodium thiosulphate) for 30 minutes or one night. After washing, the gel is placed in a silver solution (0.1% weight/volume of silver nitrate + formaldehyde) and developed (2.5% weight/volume of sodium carbonate + formaldehyde). The reaction is stopped with Na₂ EDTA (1.5% weight/volume). The gels are preserved in a glycerol solution.

- *transfer to a PVDF membrane* (Immobilon-P®, Millipore). The separated proteins are transferred to a PVDF membrane using a 100 mM CAPS buffer (Sigma) of pH 11. The transfers are incubated for one hour in TBS buffer (Tris buffer saline) with 5% of casein (milk) and 18 hours in TBS buffer (+ 1% of casein) containing antibody (1/500 anti-CV2 serum). After washing with TBS-casein (15 minutes), visualization is carried out by incubating the transfers for 1 and a half hours with biotinylated anti-IgG antibodies (1/1000) and for 1 and a half hours with the streptavidin-peroxidase complex (1/2000). The transfers are then visualized with DAB (0.06% weight/volume diaminobenzidine in 0.05 M Tris) and with H₂O₂ (0.02 µg/ml).

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A single band corresponding to a protein of 66 kDa is visible. This is specifically labelled with anti-CV2 antibodies (Figure 1). An N-terminal sequencing of this protein was then carried out, after
5 trypsin digestion.

Seven peptides, having the following sequences, were obtained:

- 1 - X-Met-Tyr-Asp-Gly-Pro
- 2 - X-Phe-Asn-Leu-Tyr-Pro-Arg
- 10 3 - X-Val-Leu-Glu-Asp-Gly-Thr-Leu-His-Val-Thr-Glu-Gly
- 4 - X-Ile-Gly-X-X-Ala-Gln-Val-(His ?)-Ala-Glu-Asn-Gly-X-Ile-Ile-Ala-Glu-Glu-Gln
- 5 - X-X-Glu-Asn-Gln-Phe-Val-Ala-Val-Thr
- 6 - X-Val-Asn-Asp-(Asp ?)-Gln-Ser-Phe-Tyr-Ala-Asp-Ile-
- 15 Tyr-Met-Glu-(Asp ?)-(Gly ?)-Leu-Ile
- 7 - X-X-X-Phe-Val-Thr-X-Pro-X-Leu-X-Pro

X: corresponds to a non-determined amino acid,

(?): corresponds to a probable but uncertain amino acid.

20 According to the analysis of databanks available in 1994, no known protein corresponded to these sequences.

EXAMPLE 2

25 Cloning of the cDNA of POP-66 or of related proteins

The cloning of the cDNA of the POP-66 protein or of related proteins was carried out by using degenerate oligonucleotide probes obtained from fragments of two peptides:

30 Ile-Ile-Ala-Glu-Glu-Gln

Tyr-Ala-Asp-Ile-Tyr-Met-Glu-(Asp ?)

Four sets of degenerate oligonucleotide primers (sense/antisense) are therefore determined

(AT(C/T)ATTGC(T/A)GA(A/G)CA;TG(C/T)TC(T/C)AC(T/A)-
35 GCAT(A/G)AT;

TATGC(A/T)GA(C/T)AT(C/T)ATGGA; TCCAT(G/A)TA(G/A)CT-(T/A)GCATA, and used for a PCR amplification.

The matrix is prepared in the form of double-stranded cDNA (Promega kit) from poly(A⁺)RNA extracted

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from the brain of rats 10 days old (Zivic-Miller, USA) using the Fast Track mRNA isolation kit (Invitrogen).

The conditions of amplification by PCR are as follows: 35 cycles at 94°C, 1 minute for denaturation, 5 55°C, 1 minute for hybridization and 72°C, two minutes, for extension.

The PCR products are analysed by 1% agarose gel electrophoresis, electroeluted, cloned in a TA cloning vector (Invitrogen) and sequenced using the primer 10 sites of the T7 and SP6 promoters.

The sequence of amino acids inferred from the MFB-17 clone agrees with the sequences of the two original peptides of POP-66 determined by the analysis of the amino acid sequence.

15 A comparative analysis of the nucleic acid sequences using the Genbank and EMBL databases reveals that MFB-17 is a partial cDNA with a nucleotide sequence identical to that of a segment of TOAD-64, a rat neuronal protein (Minturn et al., 1995).

20 The amino acid sequence inferred in the cDNA of TOAD-64 agrees with the sequences of the seven peptides determined by partial sequence analysis of the protein recognized by the anti-CV2 antibodies after purification by electrophoresis.

25 The molecular weight, the isoelectric point, the immunohistochemical profile and the regulation of TOAD-64 are similar to those of the POP-66 antigen.

30 Since the MFB-17 clone did not have the complete coding region, it was necessary to produce an intact recombinant protein to continue the research concerning the CV2 protein.

To obtain a complete TOAD-64 protein, the ds-cDNA matrix of rat brains was amplified with two sets of primers situated at the 5' and 3' extremities 35 of the coding regions

(sense: GGCATATGCTCTTATCAGGGGAAG;

antisense: GCGAATTCTTAGCCCAGGCTGATG).

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This approach allowed two different clones to be produced, one corresponding to the TOAD-64 sequence and the other to a clone designated by C-22.

EXAMPLE 3:

5 Comparison of the amino acid sequence inferred from C-22 with the ULIP proteins

10 The amino acid sequence inferred from the open reading frame indicates that this C-22 clone belongs to the superfamily of ULIP genes represented by several genes of EST sequences.

 The amino acid sequence inferred from C-22 has a homology of 30% with the amino acid sequence of the unc-33 protein of *Caenorhabditis elegans*.

15 Recently, four different homologous genes in the unc-33 protein have been described in mammals and the chicken.

 An analysis of the sequences by the Genbank databases and protein banks has allowed a classification of the unc-33- like (ULIP) proteins into
20 four different subgroups to be proposed (Byk et al. 1996).

 However, as the real functions of these proteins are not clearly known, the proposed classification is simply based on the percentage of
25 identity of amino acids. ULIP-1 is represented by a mouse "unc-33-like" phosphoprotein which has a homology of 76% with TOAD-64, Crmp-62 and Munc, a mouse sequence recently available from Genbank.

30 ULIP-2 is composed of TOAD-64, Crmp-62 and Munc which between them have a 97% amino acid identity.

 The partial human EST sequences, that is to say hcrmp-1, which have a 75% identity with ULIP-1 or ULIP-2, have been found. They belong to a third group called ULIP-3. The last group identified called ULIP-4
35 comprises r-CRMP-3 in the rat and the forms ULIP-4 in the mouse and POP-66/ULIP-4 in man.

 The comparison of the amino acid sequence of the three ULIP genes, namely TOAD-64 in the rat, Crmp-62 in the chicken and ULIP-1 in the mouse, with

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the amino acid sequence inferred from the open reading frame of the present C-22 clone, using the Clustal V alignment software program reveals that C-22 has an identity of 74% with ULIP-1, 77% with Crmp-62 and 76% with TOAD-64.

The nucleotide sequence C-22 has an identity of 97% with the partial sequence EST, hCrmp-1, and thus defines the third member of the ULIP-3 group. The TOAD-64, Crmp-62 and C-22 genes each code for a protein of 572 amino acids in length, whereas the amino acid sequence inferred from ULIP-1 gives a protein of 570 amino acids.

The analysis of the amino acid sequence of C-22 does not show any signal sequence or transmembrane domain suggesting that the product(s) of the C-22 gene could be localized in the cytoplasm of the cells.

Several consensus sites of phosphorylation by the kinase C protein (S/T X R/K) appear along the length of the product of the C-22 gene. These observations suggest that C-22 is a phosphoprotein and that the slight differences in the phosphorylation could dictate the activity or the role of different members of this family of proteins throughout the cell cycle.

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Table I: Summary of proteins having a homology with the ULIPs.

Family		Species	EMBL No.
Nematode Unc-33		Nematode	Z14146
Dihydropyrimidinase	Hu DHPase	human	D78011
	Ra DHPase	rat	D63704
	Ulip	mouse	X87817
	Hu DRP3	human	D78014
	r-CRMP-1	rat	U52102
	Hu-Ulip	human	Y07818
ULIP-1 group			
	ULIP-2	mouse	SEQ ID No. 2
	Toad-64	rat	Z46882
	CRMP-62	chicken	U17277
	Munc	mouse	X87242
	HCRMP-2	human	U17279
	Hu DRP-2	human	D78013
ULIP-2 group	r-CRMP-4	rat	U52104
	ULIP-3	mouse	SEQ ID No. 4
	HCRMP-1	human	U17278
	rCRMP-1	rat	U52102
	C-22	rat	U52095
	Hu DRP-1	human	D78012
ULIP-3 group			
	ULIP-4	mouse	SEQ ID No. 6
	POP-66/ULIP-4	man	SEQ ID No. 8
	r-CRMP-3	rat	U52103
ULIP-4 group			

5

EXAMPLE 4:

Regulation of the expression of the C-22 gene:

The evaluation of alterations in the expression of the C-22 gene could have considerable significance for the knowledge of the functional aspects of the C-22 protein.

10

Consequently, the Applicant studied the possible regulation of the expression of the C-22 gene

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in the course of development. The total RNA is extracted and separated by electrophoresis on 1% agarose gel and transferred to Nytran membrane (Duchemin et al. 1987). The transfers are hybridized with a C-22 coding sequence labelled with ^{32}P , a 0.5 mM phosphate buffer and 5% SDS at 65°C for 16 hours.

At the end of the hybridization, the transfers are washed successively three times with $2 \times \text{SSC}$, 0.1% SDS at ambient temperature, then $1 \times \text{SSC}$, 0.1% SDS at 65°C for 60 minutes, and exposed to X-rays.

Under the conditions used, a single band at 3.8 kb was detected representing the C-22 mRNA which is also the smallest transcript of the unc-33 family of genes of vertebrates. The size of the transcript remains the same during the pre- and post-natal periods.

The kinetics of the C-22 gene in the brain of rats in the course of development shows that the messenger is detectable in the course of the embryonic period on day E17. The quantity of C-22 transcripts increases up to day 7 post-natally then decreases rapidly from the second week after birth to a virtually undetectable level in the adult.

Around birth, a still unknown regulation signal is probably received, which increases the expression of the C-22 gene, this signal being temporarily linked to neuronal differentiation and to axonal development.

The mRNA of C-22 has not been able to be detected by Northern Blot analysis in several regions of the brain such as the frontal cortex, the midbrain and the thalamus in adults and rats more than two years old.

In addition, it has not been possible to detect the mRNA of C-22 in non-neuronal tissues, such as the heart, the lung, the liver and the kidney in one-week old rats and adult rats.

The data on the expression profile of the mRNA of C-22 suggests a decisive role of the C-22 protein in the development of the brain.

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EXAMPLE 5:Immunoblotting of POP-66:A - Materials and methods

5

• Transfection of the ULIPs in *E. coli*

The full-length cDNAs of rat ULIP-2 and ULIP-3 and mouse ULIP-1 and ULIP-4 were subcloned directionally in the *E. coli* pET-21a(+) expression vector after introduction of a 5'Nde I site and of a 3' EcoRI site by PCR, and the four constructs were resequenced. The expression of the target gene induced by IPTG was carried out according to the protocol of the manufacturer (Novagen).

• Production of anti-ULIP antibodies

Rabbit antibodies (anti-Pep3) are directed against the peptide ITGPEGHVLSRPEEVE (amino acids 217-232 of the sequence SEQ ID No. 8), synthesized in a multiple peptide synthesis apparatus using F-moc (432A Peptide Synthesizer SYNERGY, Applied Biosystems). The purity was checked by analysis of the sequence by HPLC and mass spectrometry. 1 mg of the synthetic peptide conjugated to limpet haemocyanin, in complete Freund's adjuvant, was used to immunize rabbits with a booster dose of 0.5 mg of bound peptide in complete Freund's adjuvant after 4 weeks. The anti-Pep3 antibodies recognized the four recombinant ULIP proteins expressed in *E. coli*.

Labelling with the anti-Pep3 antibodies was removed after preincubation with peptide 3. Controls with pre-immune rabbit sera were negative.

Anti-peptide 4 antibodies directed against the peptide LEDGTLHVTEGS were produced according to the same protocol.

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B - Results

Antibodies against four of the sequenced peptides were products. Two of the sera turned out to be of particular interest.

- 5 One contains antibodies (Ac anti-pep3) which recognize several members of the ULIP family on two-dimensional electrophoresis of protein extracts of newborn rat brain (Figure 2) and on one-dimensional electrophoresis (Figure 3). On Western Blotting,
10 another antibody (Ac anti-Pep4) recognizes a single band of 66 kDA capable of corresponding to a single member of the family (Figure 3), namely ULIP-2.

EXAMPLE 6:**Immunohistochemistry**

- 15 The tissue preparations for immunohistochemistry are obtained from newborn rat brains and from post-mortem human brains, fixed at 4°C in 4% paraformaldehyde and 0.2% picric acid diluted in phosphate buffer (0.1 M, pH = 7.4), then cryoprotected.
20 Immunocytochemistry can be carried out by the indirect immunofluorescence technique. Sections of 12 µm in thickness are prepared in a cryostat and then mounted on gelatin-covered slides, treated with 0.1% Triton X100 for 2 hours in PBS buffer and 1% bovine
25 serum albumin (BSA) and incubated for 12 h with anti-CV2 serum of patients in PBS/1% BSA at ambient temperature (1/100 dilution of the serum). After several washes with PBS/1% BSA, the sections are incubated for 2 h with a rabbit anti-human antiserum
30 conjugated to fluorescein, diluted to 1% (Dakopatts) in PBS/1% BSA. After washing in PBS, the slides are examined under the microscope. The control sections are incubated either with anti-human IgG antiserum conjugated to fluorescein alone, or PBS/1% BSA alone,
35 or the patient serum alone, or finally the control serum (patients not suffering from PNS) and antibodies conjugated to fluorescein at the same dilution.

To confirm the immunofluorescence, it is possible to use indirect labelling by immunoperoxidase.

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The frozen tissue sections fixed with paraformaldehyde are incubated with 0.3% H₂O₂ (to destroy the intrinsic peroxidase activity) and 10% normal rabbit serum (to avoid the non-specific binding of the rabbit IgG) or 1% BSA. After incubation for 12 h with patient sera diluted to 1/1000 and washing, the sections are incubated for 2 h with biotinylated rabbit anti-human IgG antiserum diluted to 1/1000 in PBS/1% BSA. The bound human IgGs are visualized by incubation with an avidin-biotin-peroxidase complex (Vectastain ABC complex, Vector) and developed with 0.05% DAB (Sigma). The control sections are obtained with sera of 15 patients without PNS according to the same protocol.

A - Localization of proteins of the ULIP family with the aid of anti-peptide antibodies:

Immunohistochemical labelling was carried out on sections of newborn and adult rat brains. The anti-peptide-3 antibody recognizes (an) antigen(s) present in several cell types on sections of newborn and adult rat brains (Fig. 4). Like the patient anti-CV2 serum, the anti-peptide-4 antibodies do not allow the demonstration of any antigen on sections of newborn rat brain although they specifically label a subpopulation of oligodendrocytes in adult rat brain (Fig. 4).

B - Expression of POP-66 in the course of the normal development of the brain:

Figure 5 shows that the proliferative nerve cells of the progenitor zones of the nervous system demonstrated by the accumulation of bromodeoxyuridine (BrdU) do not express POP-66 although the non-proliferative cells which correspond to the nerve cells in differentiation or in migration express it.

EXAMPLE 7:

35 Role of POP-66 in neuronal survival

Figure 6 allows human brain sections of healthy patients and of patients suffering from PNS to be compared. In the patients suffering from PNS and having circulating anti-CV2 antibodies, a disappearance of the

- 30 -

neurons of the dentate gyrus and of pyramidal neurons (central cell band), as well as an intense astrocytic reaction, are observed.

EXAMPLE 8:

- 5 Characterization of the POP-66 protein -
identification with ULIP-4:

Materials and methods

- 10 a) Partial purification of ULIP-1
Partially purified ULIP-1 was obtained from newborn mouse brains by three purification steps. These brains were homogenized in 4 volumes of homogenization buffer (25 mM sodium phosphate, pH 7.8, 1 mM EGTA,
15 10 µg/ml of leupeptin, 25 µg/ml of aprotinin, and 10 µg/ml of pepstatin. The homogenates were centrifuged for 10 minutes at 400 × g. The plugs were resuspended in 2 volumes of homogenization buffer, homogenized and centrifuged again. The supernatants from two
20 centrifugations were collected, sonicated and centrifuged for 1 hour at 100,000 × g. The supernatant (S2) was loaded onto a column of DEAE-Sepharose CL-6B (1.75 cm² × 26 cm) equilibrated with 100 ml of buffer A (25 mM sodium phosphate, pH 7.8, 1 mM EGTA) at a flow
25 of 30 ml per hour. The proteins were eluted in 300 ml of a 0-250 mM linear gradient of sodium chloride in buffer A and 5 ml samples were collected. The fractions containing ULIP were collected and solid ammonium sulphate was added to 20% saturation. This pool was
30 loaded onto a column of phenyl-Sepharose CL-4B (1.75 cm² × 22 cm) which had been previously equilibrated with 100 ml of buffer B (10 mM sodium phosphate, pH 7.8, 1 mM EGTA) containing 20% of saturated ammonium sulphate. The proteins were eluted
35 in a linear gradient decreasing from 20 to 0% of saturated ammonium sulphate in buffer B. The fractions containing ULIP were collected and dialysed twice against 20 volumes of buffer A. The proteins were concentrated in a small (10 ml) column of DEAE-

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Sepharose CL-6B and eluted with 400 mM sodium chloride in buffer A. The eluate was desalted on a Sephadex G-25 (NAP-10) column and concentrated to a final volume of 0.5 ml by evaporation. In the last purification step, the concentrated fraction was chromatographed in three successive steps, on two Superose 12 FPLC (Fast Protein Liquid Chromatography) columns mounted in series, in buffer C (50 mM sodium phosphate, pH 7.2, 150 mM sodium chloride) at a rate of 0.3 ml/minute. The fractions (0.6 ml) were collected and the fractions enriched in ULIP were analysed. The presence of ULIP in the successive purification steps was tested by a one-dimensional Western Blot using an anti-stathmin antibody capable of cross-reactivity. The proteins were quantified according to the method of Bradford.

b) Migration on electrophoresis gel:

A one-dimensional electrophoresis was carried out on 13% polyacrylamide gels according to the method of Laemmli. The two-dimensional PAGE electrophoreses were carried out as described above. The isoelectrofocussing gels contained 2% of total ampholines, pH 6-8 and 3-10 in a ratio of 4:1. The second dimension had been carried out on 10% acrylamide gels. The proteins had been either subjected to immunoblotting or stained with silver.

c) Western Blot analysis:

The proteins were transferred from gels to nitrocellulose in buffer containing 48 mM Tris, 39 mM glycine and 5% of methanol. The membrane was saturated with casein (2.5%) in the immunoblotting solution (12 mM Tris-HCl, pH 7.4, 160 mM NaCl, 0.1% Triton X-100) and tested with an antiserum directed against the peptide I of rat stathmin (1/10,000 dilution) or an antiserum directed against the recombinant ULIP protein (dilution 1/20,000) diluted in an immunoblotting solution containing 1% of casein. The bound antibodies were detected either with a protein A labelled with ^{125}I and autoradiographed or with anti-rabbit antibodies bound to peroxidase using the ECL kit (Amersham).

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d) Analysis of the protein sequence:

The fractions enriched in ULIP were separated on polyacrylamide gels in two dimensions. The gels are fixed for 30 minutes in 25% ethanol and 10% acetic acid and stained for 3 minutes in 0.1% amido black in 1% acetic acid and 40% methanol. The gels were decolourized in 1% acetic acid and the spots corresponding to the principal form of ULIP were cut out in these three gels, collected and digested with 2 mg/ml of Lys C endoprotease. The peptides eluted from the gel were then separated by HPLC on a DEAE-C18 column with a gradient of 0-55% of acetonitrile in 0.1% trifluoro-acetic acid. The peptides were then sequenced according to the Edman automatic degradation.

e) Expression in vitro in a mammal

1 µg of the Bluescript plasmid containing the entire cDNA coding for ULIP-1, ULIP-2, ULIP-3 or ULIP-4 was used to carry out the transcription and translation in vitro with the "Reticulocyte lysate" system (Promega) according to the protocol described by the manufacturer. 5 µg of the total transcription/translation mixture of 25 µl were analysed on electrophoresis gel in two dimensions.

Results

Neither the recombinant protein ULIP-1, nor the recombinant proteins TOAD-64 (ULIP-2) and C-22 (ULIP-3) were recognized by the anti-CV2 sera. In addition, the distribution profile of the spots corresponding to POP-66 recognized by the anti-CV2 antibodies on electrophoresis in two dimensions does not correspond to the spots recognized by the anti-ULIP-1 antibodies. However, POP-66 is a member of the ULIP family since the three POP-66 spots are recognized by the anti-pep3 Ac. POP-66 therefore corresponds to a member of the family of more basic pHi.

After translation in vitro of the four proteins (ULIP-1, 2, 3 and 4), it was shown that ULIP-4 has the same 2D electrophoretic profile as POP-66 and is recognized by the anti-CV2 antibodies (Figure 7).

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For this, the ULIP-4 protein and, as control, the ULIP-2 protein were translated *in vitro* in the presence of ^{35}S methionine from cDNA clones coding for the entire proteins. The proteins were separated by two-dimensional electrophoresis (in the presence of a brain extract providing the essential references), transferred to nitrocellulose and visualized:

- by autoradiography to localize the proteins translated *in vitro* (translation);

10 - by immunoblotting with the CV2 serum.

Figure 7 shows that the three spots from the *in vitro* translation of ULIP-4 correspond to the spots recognized by CV2. These spots are not recognized in the translation of ULIP-2.

15 The CV2 serum therefore specifically recognizes ULIP-4.

This allowed POP-66 to be identified like ULIP-4.

EXAMPLE 9:

20 Chromosomal localization of the POP-66/ULIP-4 protein

Having cloned the cDNA of human ULIP-4, it is then possible to determine the chromosomal localization of the POP-66/ULIP-4 gene by genetic mapping by *in situ* isotopic hybridization (Levy and Mattei et al., 1995).

25 *In situ* hybridization is carried out on preparations of chromosomes obtained from human lymphocytes stimulated by phytohaemagglutinin cultured for 72 hours. 5-Bromodeoxyuridine was added during the last 7 hours of culture (60 $\mu\text{g}/\text{ml}$ of medium) to ensure a post-hybridization image of chromosome bands of good quality. The clone containing an insert of 1300 base pairs coding for ULIP-4 in the Bluescript vector is labelled with tritium by nick translation with a specific activity of 1×10^8 dpm. μg^{-1} . The radiolabelled probe was hybridized in the metaphase stage at a final concentration of 200 ng per ml of hybridization solution. After covering with a Kodak NTB₂ emulsion, the slides were exposed for 20 days at

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+4°C and then developed. To avoid the shifting of the silver grains during the process, the chromosome blots were previously labelled with a Giemsa buffer solution and the metaphases were photographed. The visualization of the bands was carried out by the "Giemsa fluorochrome photolysis" (FPG) method and the metaphases were rephotographed before analysis. Of the 100 cells in metaphase examined after hybridization in situ, 246 silver grains associated with the chromosomes were counted and 54 amongst these (21.9%) were localized on chromosome 10. The distribution of the grains on this chromosome was not random: 39 out of 54 (72.2% of the latter) were localized on the q25.2-q26 region of the long arm of chromosome 10.

The POP-66/ULIP-4 gene is therefore found to be situated on chromosome 10 in the q25.2-q26 region. The loci of neurodegenerative diseases and of suppressor genes of tumours involved in different types of cancer have been localized in this chromosome region. The locus of a brain disease of early origin (infantile onset spinocerebellar ataxia) was identified in the 10q24-26 region (Varilo et al., 1996; Nikali et al., 1995). The symptoms of this recessive hereditary degenerative disease which is characterized by ataxia, neuropathy and visual atrophy are similar to those observed in patients suffering from paraneoplastic neurological syndromes with circulating anti-CV2 auto-antibodies (Honnorat et al., 1996). On the other hand, 80% of glioblastomas have mutations in this chromosome region and several suppressor loci involved in different types of tumours (prostate, kidney, small-cell lung cancer and endometrial carcinomas) are localized in this chromosome region. These data support the possibility that POP-66/ULIP-4 plays a crucial role in neurodegeneration and tumorigenesis.

In this respect, it is notable that the expression of ULIP-1 is regulated upwards in neuroblastoma cells differentiated by retinoic acid and that ULIP-1 and ULIP-3 are regulated upwards but ULIP-4

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is regulated downwards in differentiated PC12 cells in the presence of NGF, suggesting that the stop in cell growth can be linked to expression levels of the ULIP proteins.

5 **EXAMPLE 10**

Expression of ULIP proteins in transfected HeLa cells

A- Materials and methods

10 A flag (EcoRI-ATGGACTACAAGGACGACGATGACAAGG-BamHI) sequence (Kodak) was cloned in the EcoRI site of pSG5 followed by ULIP-1 (EMBL X87817), base pairs: 309-2023), ULIP-2 (Y10339, base pairs: 23-1741), ULIP-3 (Y09080, base pairs: 269-1991) or ULIP-4 (Y09079, base
15 pairs: 102-1820), respectively. The HeLa cells were cultured in DMEM media (Gibco) to which 10% of foetal calf serum (v/v) was added. The transfections were carried out by calcium phosphate precipitation (Maniatis et al., 1978). The HeLa cells were mixed with
20 5 µg of pSG5flag-ULIP-1, 2, 3 and 4 plasmids and 10 µg of pUC18. Twenty-four hours after the transfection, the HeLa cells were fixed with 4% paraformaldehyde and immunolabelled with different human sera (dilution 1/300), visualized by human anti-IgG antibodies
25 conjugated to FITC (Biosys), or anti-flag antibodies (M2, Kodak) (dilution 1/1000), visualized by anti-rabbit antibodies conjugated to Texas red (Vector).

Double immunolabelling was carried out on the HeLa cells transfected with ULIPs using anti-flag and
30 anti-Pep3 antibodies. In the cells transfected by any cDNA, 10 to 20% among them showed immunolabelling with the anti-flag antibodies visualized by the anti-mouse antibodies conjugated to Texas red.

All the transfected cells were doubly labelled
35 by antibodies directed against Pep3 and a peptide common to the four ULIPs is visualized by rabbit anti-IgG antibodies conjugated to fluorescein.

Double immunolabelling was likewise carried out on HeLa cells transfected with ULIPs using anti-flag

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and anti-CV2 antibodies. The human sera of patients suffering from PNS with circulating anti-CV2 auto-antibodies labelled the cells transfected by ULIP-4, and an anti-CV2 serum likewise labelled the cells transfected by ULIP-3. No labelling of the cells transfected by ULIP-4 was detected in the control sera of patients without cancer or neurological disease.

B) Results

After transfection of HeLa cells with cDNAs labelled by the flags of ULIP-4, 10 to 20% of the cells were strongly reactive with anti-flag antibodies and anti-Pep3 antibodies which recognize the ULIP-4s of mammals. The transfected cells were not immunolabelled with control serum of 10 neurological patients without PNS nor with rabbit pre-immune serum. On the other hand, the cells transfected with small cDNA of ULIP-4 showed an intense immunoreactivity with all the 7 tested sera of patients with circulating anti-CV2 auto-antibodies. These sera are negative on cells transfected with cDNAs of other ULIPs, with the exception of a sera which also recognized the cells transfected with ULIP-3 and a serum which also recognized the cells transfected with ULIP-1, 3 and 4. No labelling was observed on non-transfected HeLa cells, with an anti-CV2 serum.

Table 1 below shows the results of indirect immunofluorescence with different sera on HeLa cells by labelled cDNAs of members of the ULIP family.

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Table 1:

Serum No.	Neurological Symptoms	Type of Tumour	ULIP-1	ULIP-2	ULIP-3	ULIP-4
Anti-Pep3	-	-	+	+	+	+
Pre-immune	-	-	-	-	-	-
Pep3						
90-002	PCD, uveitis	UC	-	-	+	+
93-484	LE	Thymoma	-	-	-	+
94-590	LE	SCLC	-	-	-	+
95-700	PEM	SCLC	+	-	+	+
95-701	PCD	Uterine sarcoma	-	-	-	+
95-706	LE, neuropathy	SCLC	-	-	-	+
97-040	PCD	SCLC	-	-	-	+
97-103	PCD	SCLC	-	-	-	+

PCD: paraneoplastic cerebellar degeneration;

5 LE: limbic encephalitis;

PEM: paraneoplastic encephalomyelitis;

UC: undifferentiated carcinoma;

SCLC: small-cell lung carcinoma.

10

EXAMPLE 11:

Expression of POP-66/ULIP-4 and of members of the ULIP family in cancers

A - Expression of ULIP-2 and ULIP-3 in cancers:

15

1) Materials and methods: RT-PCR experiments:

The total RNA was extracted using 1 ml of RNAzol™B (Bioprobe) according to the method of Chomczynski and Sacchi. The quantity of RNA was
 20 determined by optical density measured at 260 nm and its purity was determined from the ratio of the absorbances measured at 260 and 280 nm (ratios 1.8-2.0). The integrity of the RNA preparations was additionally checked by electrophoresis on 1% agarose
 25 gel in TBE (0.45 M Tris-borate, 10 mM EDTA, pH 8). The

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specificity of the primers was analysed by comparing their sequences with the various gene databanks (EMBL and FASTA). For a relative quantification, the gene coding for G3PDH (glyceraldehyde-3-phosphate dehydrogenase, Clontech), a ubiquitous gene expressed in numerous tissues including the brain, was co-amplified with the mRNA tested as an internal standard to check the uniformity of the quantities of RNA in the samples and to test the efficacy of the reverse transcription step for different RNA samples. The primers 5', 3' and the oligonucleotides of internal probes of G3PDH were synthesized and purified by Eurogentec. The total mRNA (1 µg) was denatured (15 minutes at 65°C) and transcribed in single-stranded cDNA (1 and a half hours, 42°C) in a final volume of 20 µl of buffer (50 mM Tris HCl, 75 mM KCl, pH 8.3, Gibco BRL) containing 5 ng per µl of oligo-dT 12-18 primer (Pharmacia Biotech), 40 units of reverse transcriptase of the Moloney murine leukaemia virus (Mu-LV) (Gibco BRL), 40 units of RNAsine (Promega), 10 mM DTT (Gibco BRL) and 0.5 mM of each of the triphosphate deoxynucleotides (Promega). The cDNA samples were diluted to 1/10 in distilled water and the PCR reactions were carried out using 1 µl, 4 µl or 2 µl of cDNA sample for the messenger RNA of ULIP-2 and ULIP-3, in a buffer (50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X100, 0.4% glycerol and 800 µM NaCl, pH 9), to which was added 40 µmol of DTT, 3 mmol of MgCl₂, 0.2 mmol of each dNTP, 0.4 µM of each selected primer and 2 units of AmpliTaq DNA polymerase (Promega) in a final volume of 50 µl). The samples were then placed in a thermocycler (Biomed-Hybaid), denatured at 95°C for 5 minutes and amplified for 35 cycles (one cycle = 95°C denaturation for 65 seconds, 60°C hybridization of the primers for 45 seconds, 72°C extension for 4 minutes and 15 minutes final elongation at 72°C. The products were separated by electrophoresis on 1% Seakem agarose gel and the test bands of the RT-PCR products of expected size as well as the molecular weight marker

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scale (100 base pairs) (Promega) were visualized using ethidium bromide staining.

Composition of the oligonucleotide probes used for ULIP-3 PCR

- 5 5' ATAGAGGAGCGGATGACG (899) 3'
 GCTGTTATGGTCTTCAACTTGTCGG (1092)
 GGCCTGTTATGGTCTTCAACTTGTCG (1093)

Composition of the oligonucleotide probes used for ULIP-2 PCR

- 10 5' AGGAGGAGTGAAGACCATCG 5227) 3'
 CTTATGCCACTCGCTGATGTCC (509).

2) Results

- The RT-PCR experiments show that TOAD-64
 15 (ULIP-2) and C-22 (ULIP-3) are expressed in certain small-cell lung tumours (cf. Figure 8) and absent in others, especially in cells of patients who develop paraneoplastic neurological syndromes with a good prognosis.

20

B - Expression of ULIP-4 in cancers

1) Materials and methods

- 25 • Preparation of the RNA and RT-PCR

The total RNAs are extracted from cerebral tumours preserved in liquid nitrogen according to the conventional RNAzol™ technique (Bioprobe, France). Reverse transcription was carried out using oligo(dt)₁₈
 30 on 1 µg of total RNA and the PCR was carried out with 1/20 of the volume of the mixture for the reverse transcription (RT mix). The primers used for ULIP-4 are:

- 5'CATCTGGCTGTCGCTGCAC3', 5'GCCGCCCCCTACCAGAGACC3',
 35 and for GAPDH: 5'GGAGATTTCAGTGTGGTGG3',
 5'GGCTCTCCAGAACATCATCC3'. The cDNA was denatured at 95°C for five minutes. PCR amplification was carried out for 30 cycles. ULIP-4: 95°C, 45 sec; 62°C, 45 sec; 72°C, 45 sec. GAPDH: 95°C, 45 sec; 55°C, 45 sec; 72°C,

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45 sec. The final extension was carried out at 72°C for 5 minutes.

2) Results

5 Of the 8 glioblastoma extracts studied, 4 (50%) expressed the messenger RNA of ULIP-4. Conversely, of the 10 oligodendroglioma extracts tested, none expressed the messenger RNA of ULIP-4. This differential expression, as a function of the primitive cerebral tumour type, is in favour of a potential role of ULIP-4 in the cell proliferation of these tumours.

10 The protein POP-66/ULIP-4 as well as the proteins of the ULIP family could be expressed in the peripheral tumours (small-cell lung tumour, thymoma, cancer of the breast and of the ovary). Their presence could therefore be correlated with a prognosis. The localization of the POP-66/ULIP-4 gene on the distal part of chromosome 10 confirms this in the case of cerebral tumours.

20 Thus, the differential expression of members of the ULIP family in tumours such as small-cell lung cancer, although the corresponding ULIP gene is absent in a healthy tissue, as well as the modulation of the expression of members of the ULIP family obtained during differentiation by the HTLV1 human retrovirus of a medulloblastoma line, suggest the involvement of ULIPs in cancerous tumours.

EXAMPLE 12:

30 Production of specific antibodies of each of the human ULIP proteins

Specific peptides of each member of the ULIP family were synthesized on a multiple peptide synthesis apparatus using F-moc (432A Peptide Synthesizer SYNERGY, Applied Biosystems). The purity was checked by sequence analysis by HPLC and mass spectrometry.

These peptides are:

Specific peptide of ULIP-1: G S A R G S P T R P N (11 amino acids)

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Specific peptide of ULIP-2: S S A K T S P A K Q Q A (12 amino acids)

Specific peptide of ULIP-3: P S A K S S P S K H Q (11 amino acids)

5 Specific peptide of ULIP-4: P A R A S C P G K I S (11 amino acids).

10 1 mg of the synthetic peptide conjugated to limpet haemocyanin, in complete Freund's adjuvant, was used to immunize rabbits with a booster dose of 0.5 mg of bound peptide in complete Freund's adjuvant after 4 weeks.

The antibodies obtained specifically recognize each member protein of the ULIP family.

15 EXAMPLE 13:

Production of transgenic animals expressing ULIP-4

Drosophila fruit flies were transformed by the cDNA of human ULIP-4.

20 The cDNA of ULIP-4, previously cloned in pbluescript SK-phagemid, was excised by Kpn1 and Xba1 enzymatic double digestion. After electrophoresis on agarose gel, the cDNA fragment was purified and then cloned in pUAST, originating from pCaSpeR3, digested by
25 the restriction enzymes Kpn1 and Xba1. The 10-C plasmid results from the directional cloning of the cDNA of ULIP-4 in pUAST associated with the mini-white reporter gene. The 10-C plasmid was injected with a p-delta-2-3 helper plasmid coding for the transposase of the P
30 element active in the germinal line.

The transformed fruit flies are identified by their red eyes resulting from the expression of the mini-white gene. These lines transformed by the cDNA of ULIP-4 under the control of UASGAL4 regulatory
35 sequences allow a targeted expression of the cDNA of ULIP-4.

This production of transformed fruit flies allows the role of ULIP-4 to be studied specifically in

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different cells and its involvement in human
pathologies to be understood.

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SEQUENCE LISTING

- (1) GENERAL INFORMATION:
- 5 (i) APPLICANT:
- (A) NAME: Institut National de la Santé et
de la Recherche Médicale
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- (G) TELEPHONE: 0144236000
- (H) TELEFAX: 0145856856
- 15 (ii) TITLE OF INVENTION: Use of ULIP in PNS and
associated cancers
- (iii) NUMBER OF SEQUENCES: 8
- 20 (iv) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0,
25 Version #1.25 (EPO)
- (2) INFORMATION FOR SEQ ID NO: 1:
- (i) SEQUENCE CHARACTERISTICS:
- 30 (A) LENGTH: 1817 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- 35 (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: Mus musculus

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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AGACATATAC ATGGAAGATG GGTTCATCAA GCAAATAGGA GAAAACCTGA TTGTACCAGG	180
AGGGGTGAAG ACCATCGAAG CCCACTCCAG AATGGTGATT CCCGGAGGAA TTGACGTGCA	240
<u>TACTCGCTTC</u> <u>CAGATGCCTG</u> <u>ACCAGGGAAT</u> <u>GACATCCGCT</u> <u>GATGACTTCT</u> <u>TCCAGGGAAC</u>	300
CAAGGCGGCC CTGGCCGGGG GAACCACCAT GATCATTGAC CATGTTGTTC CTGAGCCCGG	360
GACGAGCCTA TTGGTGCCT TTGATCAGTG GAGGGAGTGG GCTGACAGCA AGTCCTGCTG	420
TGACTATTCTG CTGCACGTGG ACATCACTGA GTGGCACAAG GGCATCCAGG AGGAGATGGA	480
AGCTCTGGTG AAGGACCACG GGGTAAACTC CTTCCTCGTG TACATGGCTT TCAAAGATCG	540
ATTCCAGCTG ACGGATTCCC AGATCTATGA AGTGCTGAGC GTGATCCGGG ATATCGGTGC	600
CATAGCTCAA GTCCACGCAG AGAATGGTGA CATCATTGCT GAGGCACAGC AGAGGATCCT	660
GGATCTGGGC ATCACGGGCC CCGAGGGACA CGTGTGAGC CGGCCAGAGG AGGTCGAGGC	720
TGAAGCTGTG AACCGGTCCA TCACTATTGC CAACCAGACC AACTGCCCTC TGTATGTCAC	780
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GGGCACCAAC GGCAGTGAAG AGCGGATGTC TGTCATTGTT GATAAAGCTG TGGTCACTGG	1140
GAAGATGGAT GAGAATCAGT TTGTGGCTGT GACCAGCACC AACGCAGCCA AAGTCTTCAA	1200
CCTTTACCCC CGGAAAGGTC GCATCTCGGT GGGATCTGAT GCTGACTTGG TCATCTGGGA	1260
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CCTGGAGGAC GGCACACTTC ATGTCACTGA AGGCTCAGGA CGCTACATTC CCCGGAAGCC	1440
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CCTGCACCAG TCTGGATTCA GCTTGTCTGG TGCTCAGATT GACGACAACA TTCCCCGCCG	1680
CACCACCCAG CGCATCGTGG CACCCCTGCG TGCCCGTGCC AACATCACCA GCCTGGGCTA	1740
AAGCCCCCTAG GCCTGCAGGC CACTTGGGGA TGGGGGATGG GACACCTGAG GACATTCTGA	1800
<u>GACTTCCTTT</u> <u>CTTCCAT</u>	1817

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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 572 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

10

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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Met Ser Tyr Gln Gly Lys Lys Asn Ile Pro Pro Ile Thr Ser Asp Arg
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20      25      30
Ala Asp Ile Tyr Met Glu Asp Gly Leu Ile Lys Gln Ile Gly Glu Asn
35      40      45
Leu Ile Val Pro Gly Gly Val Lys Thr Ile Glu Ala His Ser Arg Met
50      55      60
Val Ile Pro Gly Gly Ile Asp Val His Thr Arg Phe Gln Met Pro Asp
65      70      75      80
Gln Gly Met Thr Ser Ala Asp Asp Phe Phe Gln Gly Thr Lys Ala Ala
85      90      95
Leu Ala Gly Gly Thr Thr Met Ile Ile Asp His Val Val Pro Glu Pro
100     105     110
Gly Thr Ser Leu Leu Ala Ala Phe Asp Gln Trp Arg Glu Trp Ala Asp
115     120     125
Ser Lys Ser Cys Cys Asp Tyr Ser Leu His Val Asp Ile Thr Glu Trp
130     135     140
His Lys Gly Ile Gln Glu Glu Met Glu Ala Leu Val Lys Asp His Gly
145     150     155     160
Val Asn Ser Phe Leu Val Tyr Met Ala Phe Lys Asp Arg Phe Gln Leu
165     170     175
Thr Asp Ser Gln Ile Tyr Glu Val Leu Ser Val Ile Arg Asp Ile Gly
180     185     190
Ala Ile Ala Gln Val His Ala Glu Asn Gly Asp Ile Ile Ala Glu Ala
195     200     205

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Gln Gln Arg Ile Leu Asp Leu Gly Ile Thr Gly Pro Glu Gly His Val
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 Leu Ser Arg Pro Glu Glu Val Glu Ala Glu Ala Val Asn Arg Ser Ile
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 260 265 270
 Val Val Tyr Gly Glu Pro Ile Thr Ala Ser Leu Gly Thr Asp Gly Ser
 275 280 285
 His Tyr Trp Ser Lys Asn Trp Ala Lys Ala Ala Ala Phe Val Thr Ser
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 Pro Pro Leu Ser Pro Asp Pro Thr Thr Pro Asp Phe Leu Asn Ser Leu
 305 310 315 320
 Leu Ser Cys Gly Asp Leu Gln Val Thr Gly Ser Ala His Cys Thr Phe
 325 330 335
 Asn Thr Ala Gln Lys Ala Val Gly Lys Asp Asn Phe Thr Leu Ile Pro
 340 345 350
 Glu Gly Thr Asn Gly Thr Glu Glu Arg Met Ser Val Ile Trp Asp Lys
 355 360 365
 Ala Val Val Thr Gly Lys Met Asp Glu Asn Gln Phe Val Ala Val Thr
 370 375 380
 Ser Thr Asn Ala Ala Lys Val Phe Asn Leu Tyr Pro Arg Lys Gly Arg
 385 390 395 400
 Ile Ser Val Gly Ser Asp Ala Asp Leu Val Ile Trp Asp Pro Asp Ser
 405 410 415
 Val Lys Thr Ile Ser Ala Lys Thr His Asn Ser Ala Leu Glu Tyr Asn
 420 425 430
 Ile Phe Glu Gly Met Glu Cys Arg Gly Ser Pro Leu Val Val Ile Ser
 435 440 445
 Gln Gly Lys Ile Val Leu Glu Asp Gly Thr Leu His Val Thr Glu Gly
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 485 490 495
 Gly Leu Tyr Asp Gly Pro Val Cys Glu Val Ser Val Thr Pro Lys Thr
 500 505 510
 Val Thr Pro Ala Ser Ser Ala Lys Thr Ser Pro Ala Lys Gln Gln Ala
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 Pro Pro Val Arg Asn Leu His Gln Ser Gly Phe Ser Leu Ser Gly Ala
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 Gln Ile Asp Asp Asn Ile Pro Arg Arg Thr Thr Gln Arg Ile Val Ala
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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 2297 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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 TCTCAGGAGT AGCGCCGCGG GGAGAGACCC GGGTAGAGCG CCAGGCAGAC GTTAGTTCCA 240
 GCGGCCGGGG GGAGGGCTCC AGAGGGGCCA TGTCTCATCA GGGGAAGAAG AGCATCCCGC 300
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 AGGGCACTAA AGCAGCGCTG GCAGGTGGAA CCACGATGAT CATTGACCAC GTTGTTCCTG 600
 AACCTGGGTC CAGCTTGTTG ACTTCCTTTG AGAAATGGCA CGAAGCAGCA GACACCAAAT 660
 CCTGCTGTGA CTATTCCTTC CACGTGGACA TCACAAGCTG GTATGATGGT GTTCGGGAAG 720
 AGCTGGAGGT GCTGGTGCAG GACAAAGGTG TCAACTCCTT CCAAGTCTAC ATGGCGTATA 780
 AGGACCTGTA CCAGATGTCT GACAGCCAGC TGTATGAAGC CTCACCTTC CTTAAGGGTT 840
 TGGGAGCTGT GATCTTAGTC CATGCAGAAA ATGGAGATTT GATAGCTCAG GAACAAAAAC 900
 GGATCCTGGA GATGGGCATC ACGGGTCCCG AGGGTCATGC TCTGAGCAGA CCCGAGGAGC 960
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 ACATCACCAA GGTATGAGC AAGAGTGCAG CGGACATCAT CGCACTGGCC AGGAAGAAAG 1080
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AACATGCTGC TCCTGCTCCT TCTGCCGAAT CCTCGCCTTC TAAACACCAA CCCCACCCA	1860
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TCGGTTGACC TCAGATGAGC CAGATATGCA AGAGTGAAGG ATTATGGGAA AACGTCCATT	2040
CCTTTTCCGT GTTTTGAAG CCCACAGTTT TAGTTGGTAC TGACGGAGGG GAGATTGAGC	2100
GATGCTCTTT CCTTCTCTGT TTAGGAAGAA GTGTTACTAG TGTGGTGTGT TTGCCTGGAA	2160
GTCCCTCGCC CACAGTGTGT GTTCACACCG ACTCCACCTC AGAGCATGGT GCCGTCCGTT	2220

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(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 572 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

10

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

15

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Leu Leu Ile Arg Gly Gly Arg Ile Ile Asn Asp Asp Gln Ser Phe Tyr
20          25          30
Ala Asp Val Tyr Leu Glu Asp Gly Leu Ile Lys Gln Ile Gly Glu Asn
35          40          45
Leu Ile Val Pro Gly Gly Val Lys Thr Ile Glu Ala Asn Gly Arg Met
50          55          60
Val Ile Pro Gly Gly Ile Asp Val Asn Thr Tyr Leu Gln Lys Pro Ser
65          70          75          80
Gln Gly Met Thr Ser Ala Asp Asp Phe Phe Gln Gly Thr Lys Ala Ala
85          90          95
Leu Ala Gly Gly Thr Thr Met Ile Ile Asp His Val Val Pro Glu Pro
100         105         110
Gly Ser Ser Leu Leu Thr Ser Phe Glu Lys Trp His Glu Ala Ala Asp
115         120         125
Thr Lys Ser Cys Cys Asp Tyr Ser Leu His Val Asp Ile Thr Ser Trp
130         135         140
Tyr Asp Gly Val Arg Glu Glu Leu Glu Val Leu Val Gln Asp Lys Gly
145         150         155         160
Val Asn Ser Phe Gln Val Tyr Met Ala Tyr Lys Asp Leu Tyr Gln Met
165         170         175

```

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Ser Asp Ser Gln Leu Tyr Glu Ala Phe Thr Phe Leu Lys Gly Leu Gly
 180 185 190
 Ala Val Ile Leu Val His Ala Glu Asn Gly Asp Leu Ile Ala Gln Glu
 195 200 205
 Gln Lys Arg Ile Leu Glu Met Gly Ile Thr Gly Pro Glu Gly His Ala
 210 215 220
 Leu Ser Arg Pro Glu Glu Leu Glu Ala Glu Ala Val Phe Arg Ala Ile
 225 230 235 240
 Ala Ile Ala Gly Arg Ile Asn Cys Pro Val Tyr Ile Thr Lys Val Met
 245 250 255
 Ser Lys Ser Ala Ala Asp Ile Ile Ala Leu Ala Arg Lys Lys Gly Pro
 260 265 270
 Leu Val Phe Gly Glu Pro Ile Ala Ala Ser Leu Gly Thr Asp Gly Thr
 275 280 285
 His Tyr Trp Ser Lys Asn Trp Ala Lys Ala Ala Ala Phe Val Thr Ser
 290 295 300
 Pro Pro Leu Ser Pro Asp Pro Thr Thr Pro Asp Tyr Leu Thr Ser Leu
 305 310 315 320
 Leu Ala Cys Gly Asp Leu Gln Val Thr Gly Ser Gly His Cys Pro Tyr
 325 330 335
 Ser Ile Ala Gln Lys Ala Val Gly Lys Asp Asn Phe Thr Leu Ile Pro
 340 345 350
 Glu Gly Val Asn Gly Ile Glu Glu Arg Met Thr Val Val Trp Asp Lys
 355 360 365
 Ala Val Ala Thr Gly Lys Met Asp Glu Asn Gln Phe Val Ala Val Thr
 370 375 380
 Ser Thr Asn Ala Ala Lys Ile Phe Asn Leu Tyr Pro Arg Lys Gly Arg
 385 390 395 400
 Ile Ala Val Gly Ser Asp Ala Asp Val Val Ile Trp Asp Pro Asp Lys
 405 410 415
 Met Lys Thr Ile Thr Ala Lys Ser His Lys Ser Thr Val Glu Tyr Asn
 420 425 430
 Ile Phe Glu Gly Met Glu Cys His Gly Ser Pro Leu Val Val Ile Ser
 435 440 445
 Gln Gly Lys Ile Val Phe Glu Asp Gly Asn Ile Ser Val Ser Lys Gly
 450 455 460
 Met Gly Arg Phe Ile Pro Arg Lys Pro Phe Pro Glu His Leu Tyr Gln
 465 470 475 480
 Arg Val Arg Ile Arg Ser Lys Val Phe Gly Leu His Ser Val Ser Arg
 485 490 495
 Gly Met Tyr Asp Gly Pro Val Tyr Glu Val Pro Ala Thr Pro Lys His
 500 505 510
 Ala Ala Pro Ala Pro Ser Ala Glu Ser Ser Pro Ser Lys His Gln Pro
 515 520 525
 Pro Pro Ile Arg Asn Leu His Gln Ser Asn Phe Ser Leu Ser Gly Ala
 530 535 540
 Gln Ile Asp Asp Asn Asn Pro Arg Arg Thr Gly His Arg Ile Val Ala
 545 550 555 560
 Pro Pro Gly Gly Arg Ser Asn Ile Thr Ser Leu Gly
 565 570

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(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 1920 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

10

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

15 GCTGACTAAT ATGCTTAAAT TCAGCCGGTC GCCACGTCTG GTCCGTACGT CCACGCCCGC 60
 GCAGCCCCCTA CCGAGGACAC TCAGCCCGCC CCGTATACAG GATGTCTTTC CAAGGCAAGA 120
 AGAGCAITCC CCGGATAACG AGCGACCCGC TTCTCATCAA AGTGGGAGG ATTGTGAACG 180
 ATGACCAGTC CTTTCATGCT GATCTGTATG TGGAGACGG TGTGATTAAA CAAATTGGAG 240
 AAAATCTCAT CCTCCCTGGG GGCATCAAAA CCATCGATGC TCATGCCCTG ATGCTGCTGC 300
 CTGGGGGAGT TGACGTTTAC ACCCGGCTGC AGATGCTGT GATGGGATG ACCCCAGCTG 360
 ATGATTCTG TCAGGGGACC AAGGGGGTTC TAGCAGGCGG GACCACCATG ATATTGGACC 420
 ATGTGTTTCC TGACGCTGCT GTGAGCTTGC TGGCAGCTTA TGAGCAGTGG CCGGACGGAG 480
 CAGACAGCGG GCGCTGCTGT GACTACTCCT TACATGTGGA CATTCCTCCG TGGCAGGAGA 540
 GCACCAAGA AGAGCTGGAG GCGCTACTCA GGCACAAAGG TGTGAATCC TTCTGCTCT 600
 TCATGGCATA CAAGGACAGG TGCCAGTGTG CTGACGCTCA GATATATGAA ATCTTCAGCC 660
 TCATCCGSEA CCGTGGGAGT GTGGCCGAGG TGCACGTA GAATGGGAC ATCGTGGAGG 720
 AGGAACAGAA GCGCTGCTG GAGCAAGGCA TCACTGCTCC TGAGGCCAT GTGCTCAGCC 780
 ACCCAGAAGA GTTAGAGGCC GAGGCTGTGT ACAGAGCAGT CACCATTTGC AAGCAGGCCA 840
 ACTGCCCACT ATACGTCACC AAGGTGATGA GCAAGGCTGC AGCTGACATG GTTGCCCAAG 900
 CCAAGGECAG GGGGGTGGTC GTCTTTGGGG AACCTATCAC TGCCAGCCTG GGCATGATG 960
 GCTCACACTA CTGGAGCAAG AACTGGGCCA AGGCTGAGC GTTGTCACT TCACCCCTTA 1020
 TCAACCCGGA CCGTAATACT GCAGACCAGC TCACCTCTCT GCTGTCCAGT GGGGACCTCC 1080
 AGGTGACAGG CAGTGCCAC TCACCTTCA CTACTGCCCA GAAGGCTGTT GGCAGAGACA 1140
 ACTTCAGACT GATCCCGGAG GTAGTCAAGG GTATAGAAGA GCGCATGTCT GTGCTCTGGG 1200
 AGAAATGTGT GCGTTCAGGG AAAATGGAGG AGAATGAGTT CATTGCCGTG ACCAGCACAA 1260
 ATGCTGCCAA AATCTTCAAT TTTACCCCA GGAAGGGGCG TGTGGCCGTG GGTCTGATG 1320
 CTGAGCTGCT CATCTGGAAC CCGAGGGCCA CGAAAGTCAT CTCTGCCAAG AGCCTAAGC 1380
 TGAATGTAGA GTACAACATC TTGAAGGAG TGGAGTGGG AGGAGTGGCC ACGGTGCTCA 1440
 TAAGTCAGGG CAGAGTGGTG CTGGAGGAGG GAACCTGCT TGTCACTCCA GGGGCTGGCC 1500
 GCTTCATTCC CCGGAAGAGC TTCCCGGACT TTGTCTATAA GAGGATAAAG GCTGCAACA 1560
 GGCTAGCAGA GATCCAGGCT GTGCTCGAG GCCTGTACGA CCGGCTGTG CATGAAGTGA 1620
 TGTACCTGC CAAGCCAGGA AGTGGCACAC AGGCCCGTGC ATCCTGTCTA GGCAGATCT 1680
 CAGTGCCACC CGTGGCAAC CTGCACCACT CGGGTTGAG CTTATCTGGC TCTCAGGCTG 1740
 ACCATCAGAT TGCCAGAGCT ACGGCTCAGA AGATCATGG ACCCCCCGSA GAGCGCTCCA 1800
 ACATCACGTC TCTTCTTAG ACTTGGGCTC TTGGCAAGCT GGTGCTGTCC CCACTGGCAG 1860
 GGTGTGGGGA CGACTCAGCT CAGTACGTC CTTCCTTTGT AGATTATTAT TGTAAAGGC 1920

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(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 572 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

10

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

15

```

Met Ser Phe Gln Gly Lys Lys Ser Ile Pro Arg Ile Thr Ser Asp Arg
1           5           10           15
Leu Leu Ile Lys Gly Gly Lys Ile Val Asn Asp Asp Gln Ser Phe His
20           25           30
Ala Asp Leu Tyr Val Glu Asp Gly Leu Ile Lys Gln Ile Gly Glu Asn
35           40           45
Leu Ile Val Pro Gly Gly Ile Lys Thr Ile Asp Ala His Gly Leu Met
50           55           60
Val Leu Pro Gly Gly Val Asp Val His Thr Arg Leu Gln Met Pro Val
65           70           75           80
Met Gly Met Thr Pro Ala Asp Asp Phe Cys Gln Gly Thr Lys Ala Ala
85           90           95
Leu Ala Gly Gly Thr Thr Met Ile Leu Asp His Val Phe Pro Asp Ala
100          105          110
Gly Val Ser Leu Leu Ala Ala Tyr Glu Gln Trp Arg Asp Gly Ala Asp
115          120          125
Ser Ala Ala Cys Cys Asp Tyr Ser Leu His Val Asp Ile Pro Arg Trp
130          135          140
His Glu Ser Thr Lys Glu Glu Leu Glu Ala Leu Val Arg Asp Lys Gly
145          150          155          160
Val Asn Ser Phe Leu Val Phe Met Ala Tyr Lys Asp Arg Cys Gln Cys
165          170          175
Thr Asp Gly Gln Ile Tyr Glu Ile Phe Ser Leu Ile Arg Asp Leu Gly
180          185          190
Ala Val Ala Gln Val His Ala Glu Asn Gly Asp Ile Val Glu Glu Glu
195          200          205

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Thr Lys Val Ile Ser Ala Lys Ser His Asn Leu Asn Val Glu Tyr Asn
420 425 430

Ile Phe Glu Gly Val Glu Cys Arg Gly Val Pro Thr Val Val Ile Ser
435 440 445

Gln Gly Arg Val Val Leu Glu Asp Gly Asn Leu Leu Val Thr Pro Gly
450 455 460

Ala Gly Arg Phe Ile Pro Arg Lys Thr Phe Pro Asp Phe Val Tyr Lys
465 470 475 480

Arg Ile Lys Ala Arg Asn Arg Leu Ala Glu Ile His Gly Val Pro Arg
485 490 495

Gly Leu Tyr Asp Gly Pro Val His Glu Val Met Leu Pro Ala Lys Pro
500 505 510

Gly Ser Gly Thr Gln Ala Arg Ala Ser Cys Ser Gly Lys Ile Ser Val
515 520 525

Pro Pro Val Arg Asn Leu His Gln Ser Gly Phe Ser Leu Ser Gly Ser
530 535 540

Gln Ala Asp Asp His Ile Ala Arg Arg Thr Ala Gln Lys Ile Met Ala
545 550 555 560

Pro Pro Gly Gly Arg Ser Asn Ile Thr Ser Leu Ser
565 570

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INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 1690 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

10

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

15

Gln Lys Arg Leu Leu Glu Gln Gly Ile Thr Gly Pro Glu Gly His Val
 210 215 220

Leu Ser His Pro Glu Glu Val Glu Ala Glu Ala Val Tyr Arg Ala Val
 225 230 235 240

Thr Ile Ala Lys Gln Ala Asn Cys Pro Leu Tyr Val Thr Lys Val Met
 245 250 255

Ser Lys Gly Ala Ala Asp Met Val Ala Gln Ala Lys Arg Arg Gly Val
 260 265 270

Val Val Phe Gly Glu Pro Ile Thr Ala Ser Leu Gly Thr Asp Gly Ser
 275 280 285

His Tyr Trp Ser Lys Asn Trp Ala Lys Ala Ala Ala Phe Val Thr Ser
 290 295 300

Pro Pro Ile Asn Pro Asp Pro Thr Thr Ala Asp His Leu Thr Ser Leu
 305 310 315 320

Leu Ser Ser Gly Asp Leu Gln Val Thr Gly Ser Ala His Cys Thr Phe
 325 330 335

Thr Thr Ala Gln Lys Ala Val Gly Lys Asp Asn Phe Thr Leu Ile Pro
 340 345 350

Glu Val Val Asn Gly Ile Glu Glu Arg Met Ser Val Val Trp Glu Lys
 355 360 365

Cys Val Ala Ser Gly Lys Met Asp Glu Asn Glu Phe Val Ala Val Thr
 370 375 380

Ser Thr Asn Ala Ala Lys Ile Phe Asn Phe Tyr Pro Arg Lys Gly Arg
 385 390 395 400

Val Ala Val Gly Ser Asp Ala Asp Leu Val Ile Trp Asn Pro Arg Ala
 405 410 415

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CCGGTTGCTG GAGCTCGGCA TCACTGGCCC CGAGGGCCAC GTGCTCAGCC ACCCCGAGGA	720
GGTGGAGGCT GAGGCGGTGT ACCGAGCTGT CACCATCGCC AAGCAGGCAA ACTGCCCGCT	780
GTACGTCACC AAGGTGATGA GCAAGGGGGC GGCCGACGCC ATCGCTCAGG CCAAGCGCAG	840
AGGGGTGGTC GTGTTTGGGG AGCCCATCAC CGCCAGCCTG GGCACCGACG GTTCACACTA	900
CTGGAGCAAG AACTGGGCCA AGGCTGCAGC CTTCGTCACA TCACCCCTG TCAACCCAGA	960
CCCCACCAGC GCAGACCACC TCACCTGCTT GCTGTCCAGC GGGGACCTCC AGGTGACAGG	1020
CAGCGCCAC TGCACCTTCA CCACTGCCCA GAAGGCTGTG GSCAAGGACA ACTTCGCGCT	1080
GATCCCCGAG GGCACCAACG GCATTGAGGA GCGCATGTG ATGCTCTGGG AGAAATGTGT	1140
GGCTCTGGG AAGATGGAGC AGAATGAGTT CGTCGCGGTG ACCAGTACAA ATGCTGCCAA	1200
AATCTTCAAT TTTTACCCAA GGAAGGGGCG AGTGGCTGTG GGCTCTGACG CTGACCTGGT	1260
CATATGGAAC CCCAAGGCCA CCAAGATCAT CTCTGCCAAG ACCCACAATC TGAACGTGGA	1320
GTACAACATC TTCGAGGGAG TGGAGTGCCG GGGAGCGCCT GCCGTGGTCA TAAGTCAGGG	1380
CCGAGTGGCG CTGGAGGACG GGAAGATGTT TGTACCCCG GGGCGGGGCC GCTTCGTCCC	1440
TCGGAAAACA TTCCCGGACT TTGTCTACAA GAGGATCAA GCTCGCAACA GGCTGGCGGA	1500
GATCCACGGT GTGCCCCGTG GGCTGTATGA CGGGCCCGTC CACGAGGTGA TGGTGCTGC	1560
CAAGCCAGGG AGTGGCGCTC CGGCCCCGCG GTCTTGCCCA GGCAAGATCT CGTGCCCTCC	1620
TGTGGGCAAC CTACATCAGT CGGGGTTGAG CCTATCTGGG TCTCAGGCTG ATGACCACAT	1680
<u>CGCCCGACGC</u>	1690

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 ID 30033145/43406
 P.14

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INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 572 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

10

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

15 Met Ser Phe Gln Gly Lys Lys Ser Ile Pro Arg Ile Thr Ser Asp Arg
 1 5 10 15
 Leu Leu Ile Arg Gly Gly Arg Ile Val Asn Asp Asp Gln Ser Phe Tyr
 20 25 30
 Ala Asp Val His Val Glu Asp Gly Leu Ile Lys Gln Ile Gly Glu Asn
 35 40 45
 Leu Ile Val Pro Gly Gly Ile His Thr Ile Asp Ala His Gly Leu Met
 50 55 60
 Val Leu Pro Gly Gly Val Asp Val His Thr Arg Leu Gln Met Pro Val
 65 70 75 80
 Leu Gly Met Thr Pro Ala Asp Asp Phe Cys Gln Gly Thr Lys Ala Ala
 85 90 95
 Leu Ala Gly Gly Thr Thr Met Ile Leu Asp His Val Phe Pro Asp Thr
 100 105 110
 Gly Val Ser Leu Leu Ala Ala Tyr Glu Gln Trp Arg Glu Arg Ala Asp
 115 120 125
 Ser Ala Ala Cys Cys Asp Tyr Ser Leu His Val Asp Ile Thr Arg Trp
 130 135 140
 His Glu Ser Ile Lys Glu Glu Leu Glu Ala Leu Val Lys Glu Lys Gly
 145 150 155 160
 Val Asn Ser Phe Leu Val Phe Met Ala Tyr Lys Asp Arg Cys Gln Cys
 165 170 175
 Ser Asp Ser Gln Met Tyr Glu Ile Phe Ser Ile Ile Arg Asp Leu Gly
 180 185 190
 Ala Leu Ala Gln Val His Ala Glu Asn Gly Asp Ile Val Glu Glu Glu
 195 200 205
 Gln Lys Arg Leu Leu Glu Leu Gly Ile Thr Gly Pro Glu Gly His Val
 210 215 220

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Leu Ser His Pro Glu Glu Val Glu Ala Glu Ala Val Tyr Arg Ala Val
225 230 235 240

Thr Ile Ala Lys Gln Ala Asn Cys Pro Leu Tyr Val Thr Lys Val Met
245 250 255

Ser Lys Gly Ala Ala Asp Ala Ile Ala Gln Ala Lys Arg Arg Gly Val
260 265 270

Val Val Phe Gly Glu Pro Ile Thr Ala Ser Leu Gly Thr Asp Gly Ser
275 280 285

His Tyr Trp Ser Lys Asn Trp Ala Lys Ala Ala Ala Phe Val Thr Ser
290 295 300

Pro Pro Val Asn Pro Asp Pro Thr Thr Ala Asp His Leu Thr Cys Leu
305 310 315 320

Leu Ser Ser Gly Asp Leu Gln Val Thr Gly Ser Ala His Cys Thr Phe
325 330 335

Thr Thr Ala Gln Lys Ala Val Gly Lys Asp Asn Phe Ala Leu Ile Pro
340 345 350

Glu Gly Thr Asn Gly Ile Glu Glu Arg Met Ser Met Val Trp Glu Lys
355 360 365

Cys Val Ala Ser Gly Lys Met Asp Glu Asn Glu Phe Val Ala Val Thr
370 375 380

Ser Thr Asn Ala Ala Lys Ile Phe Asn Phe Tyr Pro Arg Lys Gly Arg
385 390 395 400

Val Ala Val Gly Ser Asp Ala Asp Leu Val Ile Trp Asn Pro Lys Ala
405 410 415

Thr Lys Ile Ile Ser Ala Lys Thr His Asn Leu Asn Val Glu Tyr Asn
420 425 430

Ile Phe Glu Gly Val Glu Cys Arg Gly Ala Pro Ala Val Val Ile Ser
435 440 445

Gln Gly Arg Val Ala Leu Glu Asp Gly Lys Met Phe Val Thr Pro Gly
450 455 460

Ala Gly Arg Phe Val Pro Arg Lys Thr Phe Pro Asp Phe Val Tyr Lys
465 470 475 480

Arg Ile Lys Ala Arg Asn Arg Leu Ala Glu Ile His Gly Val Pro Arg
485 490 495

Gly Leu Tyr Asp Gly Pro Val His Glu Val Met Val Pro Ala Lys Pro
500 505 510

Gly Ser Gly Ala Pro Ala Arg Ala Ser Cys Pro Gly Lys Ile Ser Val
515 520 525

Pro Pro Val Arg Asn Leu His Gln Ser Gly Phe Ser Leu Ser Gly Ser
530 535 540

Gln Ala Asp Asp His Ile Ala Arg Arg Thr Ala Gln Lys Ile Met Ala
545 550 555 560

Pro Pro Gly Gly Arg Ser Asn Ile Thr Ser Leu Ser
565 570

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PCT/FR98/00328

CLAIMS

1. Purified polypeptide, derivative or polypeptide
fragment of the said biologically active purified
5 polypeptide, comprising an amino acid sequence selected
from SEQ ID No. 2, No. 4, No. 6 and No. 8.
2. Purified polypeptide, derivative or
biologically active polypeptide fragment of the said
purified polypeptide, according to Claim 1, comprising
10 the amino acid sequence SEQ ID No. 8, the said
polypeptide being designated by "POP-66".
3. Isolated nucleotide sequence, comprising:
 - a sequence selected from SEQ ID No. 1, No. 3,
No. 5 and No. 7 coding for a polypeptide of amino acid
15 sequence SEQ ID No. 2, No. 4, No. 6 and No. 8
respectively;
 - a sequence derived from a sequence selected
from SEQ ID No. 1, No. 3, No. 5 and No. 7 on account of
the degeneracy of the genetic code, of mutation, of
20 deletion or of insertion;
 - or a sequence capable of hybridizing
specifically with the sequence SEQ ID No. 1, No. 3,
No. 5 or No. 7.
4. Nucleotide sequence according to Claim 3,
25 comprising the nucleotide sequence SEQ ID No. 7 coding
for a polypeptide according to Claim 2.
5. Cloning and/or expression vector containing a
nucleic acid sequence according to one of Claims 3 and
4.
- 30 6. Host cell transfected by a vector according to
Claim 5.
7. Mono- or polyclonal antibodies obtained from a
purified polypeptide according to one of Claims 1 and
2, a derivative or a biologically active polypeptide
35 fragment of the said purified polypeptide, as well as
the fragments, the chimeric antibodies or the
immunoconjugates of the said mono- or polyclonal
antibodies.

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8. Composition useful for the diagnosis of paraneoplastic neurological syndromes and/or for the early diagnosis of the formation of tumours, characterized in that it comprises a purified POP-66 polypeptide, derivative or biologically active polypeptide fragment of POP-66 according to Claim 2.

9. Use of a purified POP-66 polypeptide, derivative or biologically active polypeptide fragment of POP-66 according to Claim 2 or of a nucleotide sequence according to Claim 4 for detecting the presence of anti-CV2 antibodies in a biological sample.

10. Use of mono- or polyclonal antibodies or their fragments, chimeric or immunoconjugated antibodies according to Claim 7 for the purification or the detection of a corresponding ULIP protein in a biological sample.

11. Use of antibodies directed against a protein of the ULIP family for the demonstration of a ULIP protein in neoplasms and paraneoplastic neurological syndromes, for diagnostic purposes.

12. Use according to Claim 11, the antibodies being monoclonal antibodies obtained from polyclonal anti-CV2 serum of patients.

13. Method for the diagnosis of paraneoplastic neurological syndromes and/or for the early diagnosis of the formation of cancerous tumours, characterized in that auto-antibodies directed against a POP-66 protein are demonstrated in a blood sample taken from an individual by

- the contacting of a blood sample taken from an individual with a purified polypeptide (POP-66), derivative or biologically active polypeptide fragment of POP-66 according to Claim 2, optionally attached to a support under conditions allowing the formation of specific immunological complexes between the said polypeptide and the auto-antibodies optionally present in the blood sample, and

- the detection of the specific immunological complexes optionally formed.

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14. Kit for the diagnosis of paraneoplastic neurological syndromes and for the early diagnosis of the formation of tumours from a biological sample, comprising:

- 5 - at least one purified POP-66 polypeptide, derivative or biologically active polypeptide fragment of POP-66, according to Claim 2, optionally attached to a support,
- means of visualization of the formation of
- 10 specific antigen/antibody complexes between an anti-POP-66 auto-antibody and the said purified POP-66 polypeptide, derivative or polypeptide fragment and/or means of quantification of these complexes.

15. Pharmaceutical composition, comprising at least
- 15 one purified protein of the ULIP family, polypeptide fragment or biologically active derivative of this, a nucleotide sequence or nucleotide sequence fragment coding for the said protein, an antisense sequence capable of hybridizing specifically with a nucleotide
- 20 sequence coding for the said protein, or an antibody directed against the said protein, combined with a pharmaceutically acceptable vehicle.

16. Pharmaceutical composition according to Claim
- 15, comprising at least one purified POP-66 polypeptide
- 25 according to Claim 2, polypeptide fragment or biologically active derivative of this, a nucleotide sequence or nucleotide sequence fragment coding for the said polypeptide, an antisense sequence capable of hybridizing specifically with a nucleotide sequence
- 30 coding for the said polypeptide, or an antibody directed against the said polypeptide, combined with a pharmaceutically acceptable vehicle.

17. Use of a purified protein of the ULIP family, polypeptide fragment or biologically active derivative
- 35 of this, a nucleotide sequence or nucleotide sequence fragment coding for the said protein, an antisense sequence capable of hybridizing specifically with a nucleotide sequence coding for the said protein, or an antibody directed against the said protein, for the

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production of a medicament intended for treating neurodegenerative illnesses and neoplasms.

18. Method of treatment of neurodegenerative illnesses and neoplasms, comprising the administration
5 to a subject requiring such a treatment of a therapeutically efficacious quantity of a purified protein of the ULIP family, polypeptide fragment or biologically active derivative of this, a nucleotide
10 sequence or nucleotide sequence fragment coding for the said protein, an antisense sequence capable of hybridizing specifically with a nucleotide sequence coding for the said protein, or an antibody directed against the said protein, combined with a pharmaceutically acceptable vehicle.

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CLAIMS

1. Purified polypeptide comprising an amino acid sequence selected from SEQ ID No. 2, No. 4, No. 6 and
5 No. 8.
2. Purified polypeptide according to Claim 1, comprising the amino acid sequence SEQ ID No. 8, the said polypeptide being designated by "POP-66".
3. Isolated nucleotide acid comprising a sequence
10 coding for a polypeptide of amino acid sequence SEQ ID No. 2, No. 4, No. 6 or No. 8.
4. Nucleic acid according to Claim 3, comprising a sequence selected from SEQ ID No. 1, No. 3, No. 5 or No. 7, respectively coding for a polypeptide of amino
15 acid sequence SEQ ID No. 2, No. 4, No. 6 or No. 8.
5. Nucleic acid according to Claim 4, comprising the nucleotide sequence SEQ ID No. 7 coding for a polypeptide according to Claim 2.
6. Cloning and/or expression vector containing a
20 nucleic acid sequence according to one of Claims 3 to 5.
7. Host cell transfected by a vector according to Claim 6.
8. Mono- or polyclonal antibodies obtained from a
25 purified polypeptide according to one of Claims 1 and 2, as well as the fragments, the chimeric antibodies or the immunoconjugates of the said mono- or polyclonal antibodies.
9. Composition useful for the diagnosis of
30 paraneoplastic neurological syndromes and/or for the early diagnosis of the formation of tumours, characterized in that it comprises a purified POP-66 polypeptide, according to Claim 2.
10. Use of a purified POP-66 polypeptide according
35 to Claim 2, derivative or biologically active polypeptide fragment of POP-66, or of a nucleic acid according to Claim 5 for detecting the presence of anti-CV2 antibodies in a biological sample.

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11. Use of mono- or polyclonal antibodies or their fragments, chimeric or immunoconjugated antibodies according to Claim 8 for the purification or the detection of a corresponding ULIP protein in a biological sample.

12. Use of antibodies directed against a protein of the ULIP family for the demonstration of a ULIP protein in neoplasms and paraneoplastic neurological syndromes, for diagnostic purposes.

13. Use according to Claim 12, the antibodies being monoclonal antibodies obtained from polyclonal anti-CV2 serum of patients.

14. Method for the diagnosis of paraneoplastic neurological syndromes and/or for the early diagnosis of the formation of cancerous tumours, characterized in that auto-antibodies directed against a POP-66 protein are demonstrated in a blood sample taken from an individual by

- the contacting of a blood sample taken from an individual with a purified polypeptide (POP-66) according to Claim 2, derivative or biologically active polypeptide fragment of POP-66, optionally attached to a support under conditions allowing the formation of specific immunological complexes between the said polypeptide and the auto-antibodies optionally present in the blood sample, and

- the detection of the specific immunological complexes optionally formed.

15. Kit for the diagnosis of paraneoplastic neurological syndromes and for the early diagnosis of the formation of tumours from a biological sample, comprising:

- at least one purified POP-66, according to Claim 2, derivative or biologically active polypeptide fragment of POP-66 polypeptide, optionally attached to a support,

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- means of visualization of the formation of specific antigen/antibody complexes between an anti-POP-66 auto-antibody and the said purified POP-66 polypeptide, derivative or polypeptide fragment and/or means of quantification of these complexes.

16. Pharmaceutical composition, comprising at least one purified protein of the ULIP family, polypeptide fragment or biologically active derivative of this, a nucleotide sequence or nucleotide sequence fragment coding for the said protein, an antisense sequence capable of hybridizing specifically with a nucleotide sequence coding for the said protein, or an antibody directed against the said protein, combined with a pharmaceutically acceptable vehicle.

17. Pharmaceutical composition according to Claim 15, comprising at least one purified POP-66 polypeptide according to Claim 2, polypeptide fragment or biologically active derivative of this, a nucleotide sequence or nucleotide sequence fragment coding for the said polypeptide, an antisense sequence capable of hybridizing specifically with a nucleotide sequence coding for the said polypeptide, or an antibody directed against the said polypeptide, combined with a pharmaceutically acceptable vehicle.

18. Use of a purified protein of the ULIP family, polypeptide fragment or biologically active derivative of this, a nucleotide sequence or nucleotide sequence fragment coding for the said protein, an antisense sequence capable of hybridizing specifically with a nucleotide sequence coding for the said protein, or an antibody directed against the said protein, for the production of a medicament intended for treating neurodegenerative illnesses and neoplasms.

19. Method of treatment of neurodegenerative illnesses and neoplasms, comprising the administration to a subject requiring such a treatment of a therapeutically efficacious quantity of a purified

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protein of the ULIP family, polypeptide fragment or
biologically active derivative of this, a nucleotide
sequence or nucleotide sequence fragment coding for the
said protein, an antisense sequence capable of
5 hybridizing specifically with a nucleotide sequence
coding for the said protein, or an antibody directed
against the said protein, combined with a
pharmaceutically acceptable vehicle.

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TOTAL P.25

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Molecular
weight scale +

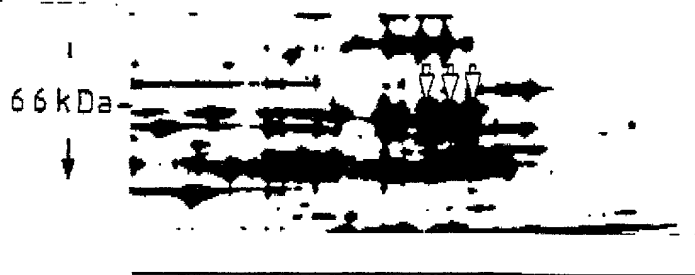


FIG. 1A

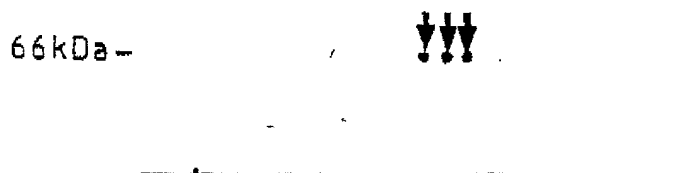
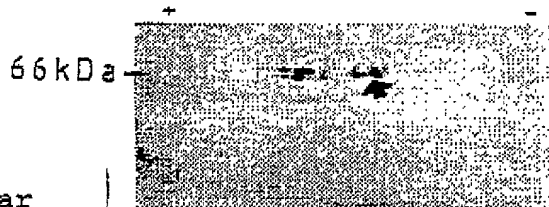


FIG. 1B

Molecular
weight scale



ULIP family

FIG. 2A



CV₂ antigen (POP 66/ULIP4)

FIG. 2B

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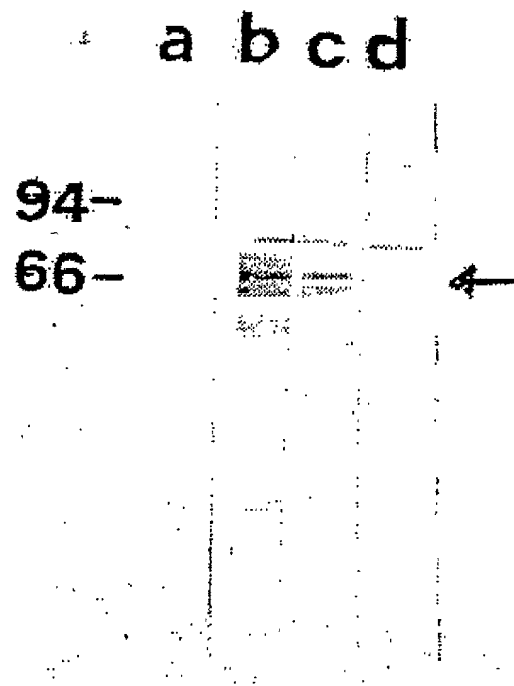


FIG. 3

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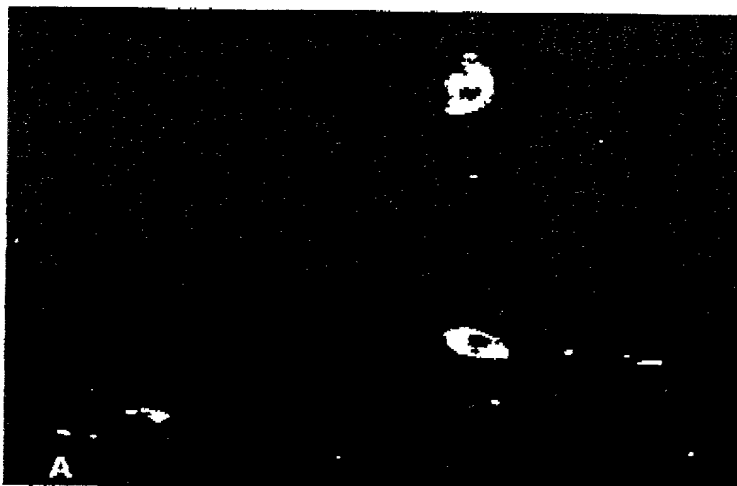


FIG. 4A



FIG. 4B

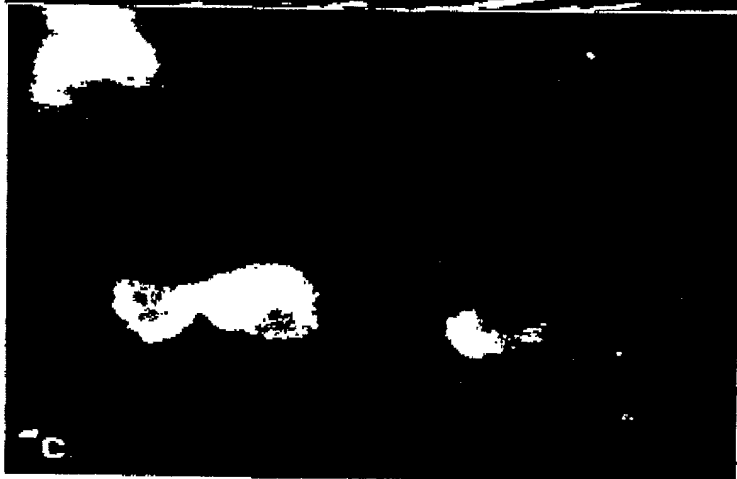


FIG. 4C

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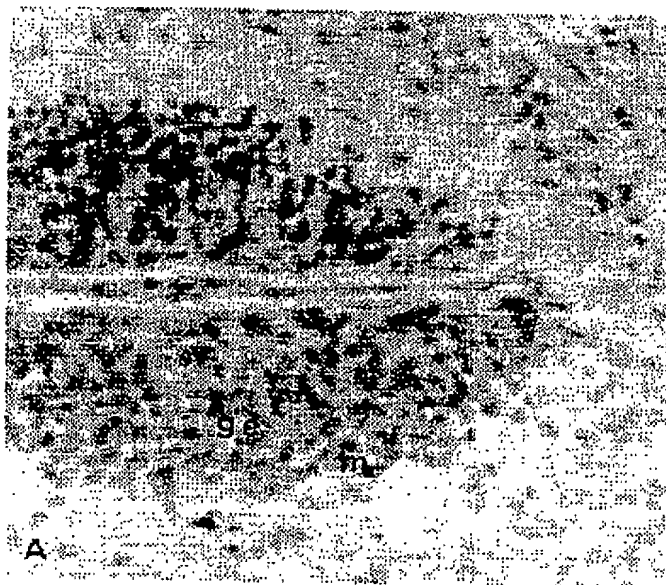


FIG. 5A

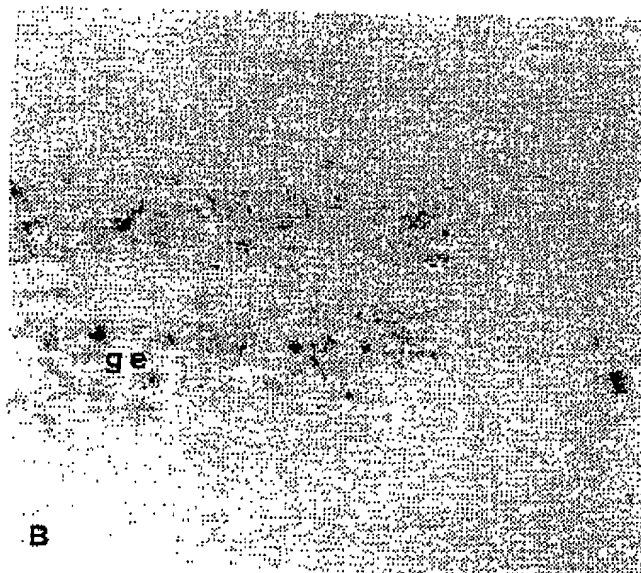


FIG. 5B

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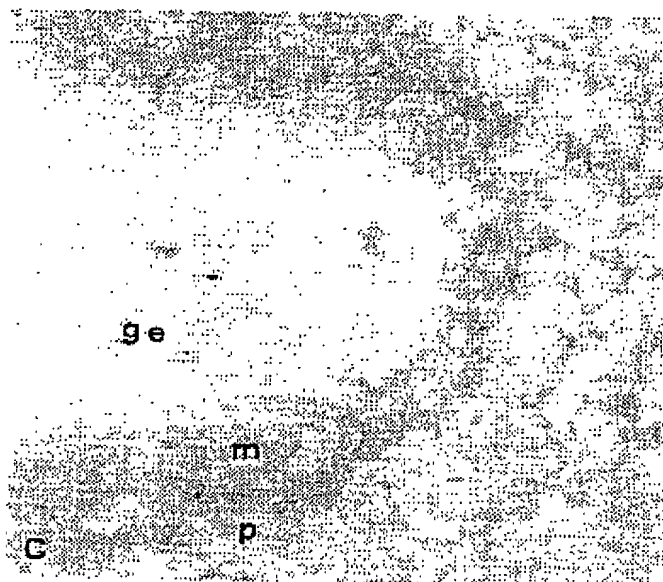


FIG. 5C

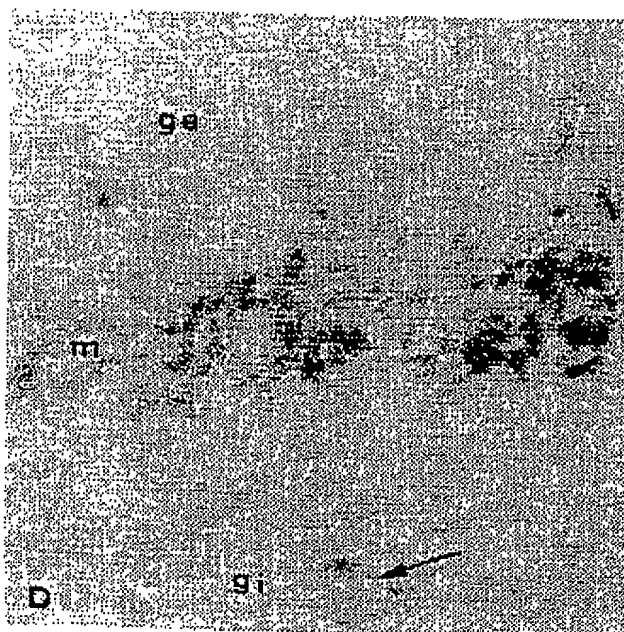


FIG. 5D

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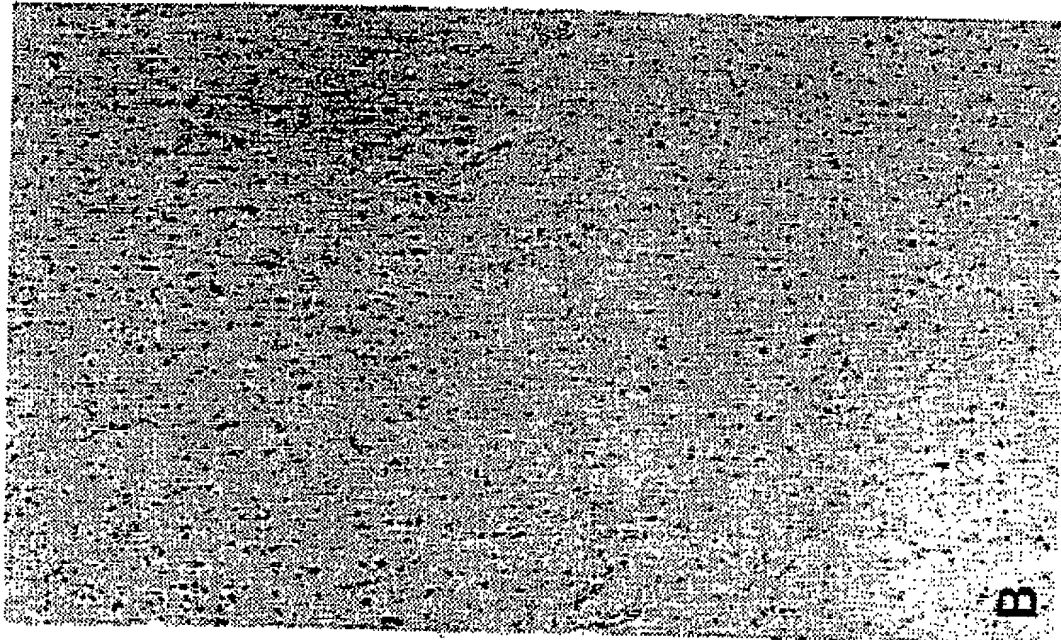


FIG. 6B

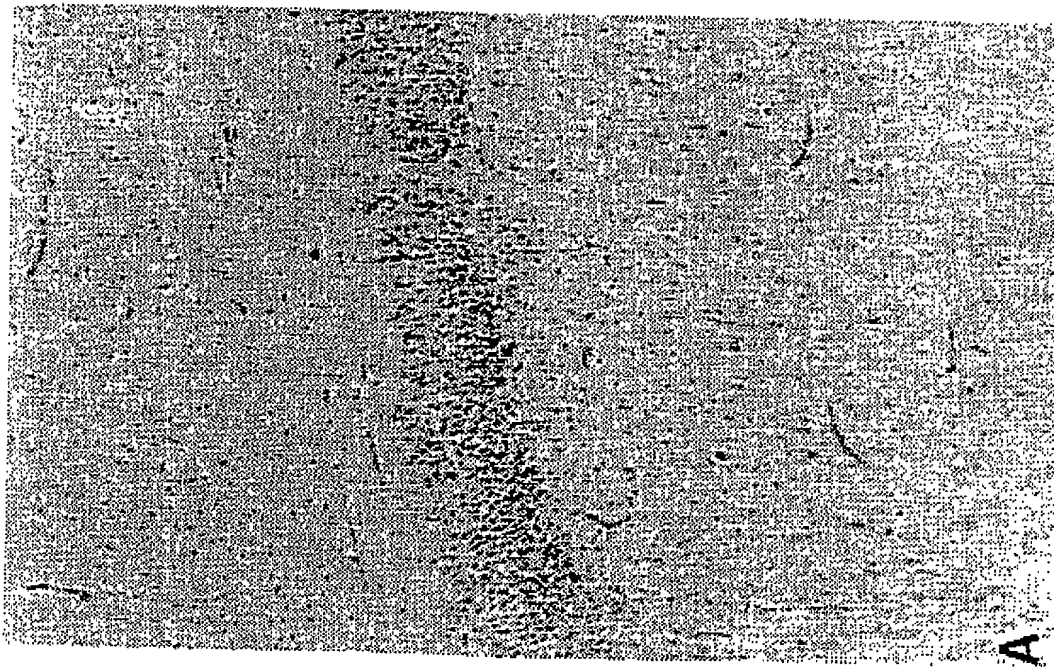


FIG. 6A

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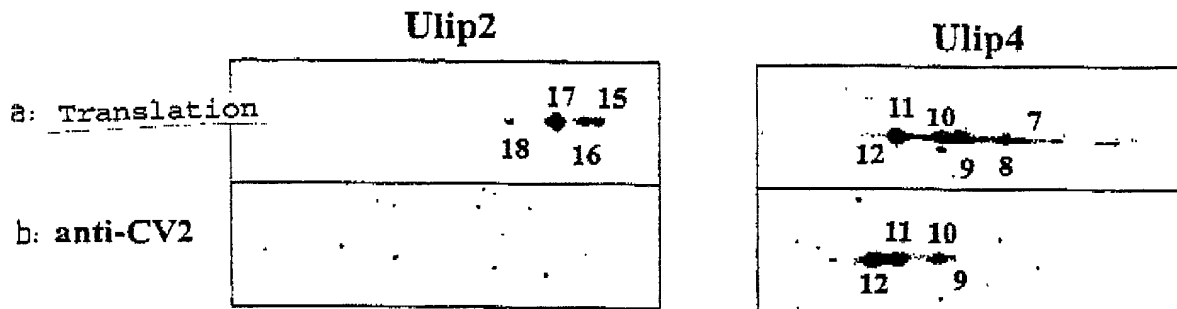


FIG. 7A

FIG. 7B

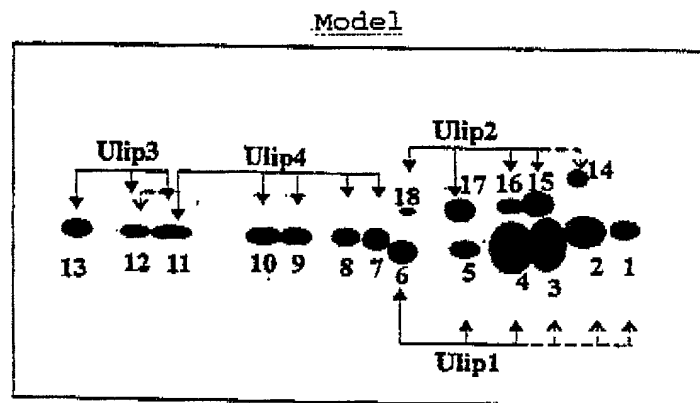


FIG. 7C

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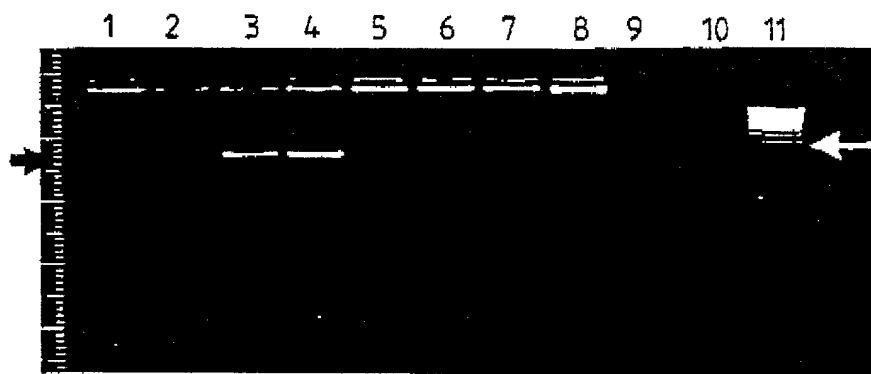


FIG. 8A

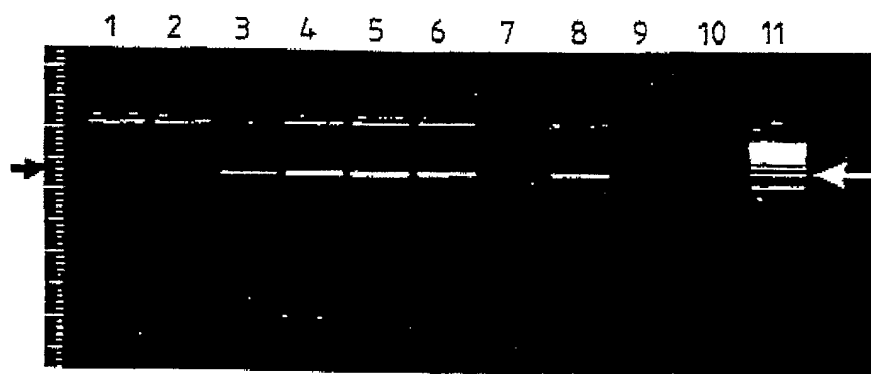


FIG. 8B

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ULIP2 mouse

cttctcctccgcccccgagag	ATG TCT TAT CAG GGG AAG AAA AAT ATT CCA CCC ATC	I P P I	12 58
T S D R L I K G G K I V N D D Q S	ACG AGC GAT CGT CTT CTG ATC AAA GGT GGC AAG ATT GTG AAT GAT GAC CAG TCC		30 112
F Y A D I Y M E D G L I K Q I G E N	TTC TAT GCA GAC ATA TAC ATG GAA GAT GGG TTG ATC AAG CAA ATA GGA GAA AAC		48 166
L I V P G G V K T I E A H S R M V I	CTG ATT GTA CCA GGA GGG GTG AAG ACC ATC GAA GCC CAC TCC AGA ATG GTG ATT		66 220
P G G I D V H T R F Q M P D Q G M T	CCC GGA GGA ATT GAC GTG CAT ACT CGC TTC CAG ATG CCT GAC CAG GGA ATG ACA		84 274
S A D D F F Q G T K A A L A G G T T	TCC GCT GAT GAC TTC TTC CAG GGA ACC AAG GCG GCC CTC GGC GGA ACC ACC		102 328
M I I D H V V P E P G T S L L A A F	ATG ATC ATT GAC CAT GTT GTT CCT GAG CCC GGG ACG AGC CTA TTG GCT GCC TTT		120 382
D Q W R E W A D S K S C C D Y S L H	GAT CAG TGG AGG GAG TGG GCT GAC AGC AAG TCC TGC TGT GAC TAT TCG CTG CAC		138 436
V D I T E W H K G I Q E E M E A L V	GTG GAC ATC ACT GAG TGG CAC AAG GGC ATC CAG GAG ATG GAA GCT CTG GTG		156 490

FIG. 9

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K D H G V N S F L V Y M A F K D R F 174
AAG GAC CAC GGG GTA AAC TCC TTC CTC GTG TAC ATG GCT TTC AAA GAT CGA TTC 544

Q L T D S Q I Y E V L S V I R D I G 192
CAG CTG ACG GAT TCC CAG ATC TAT GAA GTG CTG AGC GTG ATC CGG GAT ATC GGT 598

A I A Q V H A E N G D I I A E A Q Q 210
GCC ATA GCT CAA GTC CAC GCA GAG AAT GGT GAC ATC ATT GCT GAG GCA CAG CAG 652

R I L D L G I T G P E G H V L S R P 228
AGG ATC CTG GAT CTG GGC ATC ACG GGC CCC GAG GGA CAC GTG TTG AGC CGG CCA 706

E E V E A E A V N R S I T I A N Q T 246
GAG GAG GTC GAG GCT GAA GCT GTG AAC CGG TCC ATC ACT ATT GCC AAC CAG ACC 760

N C P L Y V T K V M P K S A A E V I 264
AAC TGC CCT CTG TAT GTC ACC AAA GTC ATG CCC AAG AGT GCG GCT GAA GTC ATC 814

A Q A R K K G T V V Y G E P I T A S 282
GCT CAG GCA CGG AAG AAG GGA ACT GTG GTG TAT GGT GAG CCC ATC ACG GCC AGC 868

L G T D G S H Y W S K N W A K A A A 300
CTG GGG ACT GAT GGC TCT CAT TAC TGG AGC AAG AAC TGG GCC AAG GCT GCG GCC 922

F V T S P P L S P D P T T P D F L N 318
TTT GTC ACC TCC CCA CCC TTG AGC CCC GAC CCA ACC ACT CCA GAC TTT CTC AAC 976

S L L S C G D L Q V T G S A H C T F 336
TCG TTG CTG TCC TGT GGA GAC CTC CAA GTC ACT GGC AGT GCC CAC TGC ACC TTC 1030

FIG. 9 continued

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N T A Q K A V G K D N F T L I P E G 354
 AAC ACT GCC CAG AAG GCT GTG GGG AAG GAC AAC TTC ACC TTG ATT CCC GAG GGC 1084

 T N G T E E R M S V I W D K A V V T 372
 ACC AAC GGC ACT GAG GAG CGG ATG TCT GTG GAT AAA GCT GTG GTC ACT 1138

 G K M D E N Q F V A V T S T N A A K 390
 GGG AAG ATG GAT GAG AAT CAG TTT GTG GCT GTG ACC AGC ACC AAC GCA GCC AAA 1192

 V F N L Y P R K G R I S V G S D A D 408
 GTC TTC AAC CTT TAC CCC CGG AAA GGT CGC ATC TCG GTG GGA TCT GAT GCT GAC 1246

 L V I W D P D S V K T I S A K T H N 426
 TTG GTC ATC TGG GAC CCT GAC AGT GTG AAG ACC ATC TCT GCC AAG ACA CAC AAC 1300

 S A L E Y N I F E G M E C R G S P L 444
 AGT GCT CTT GAG TAC AAC ATC TTT GAA GGC ATG GAG TGT CGC GGC TCC CCA CTG 1354

 V V I S Q G K I V L E D G T L H V T 462
 GTG GTC ATC AGC CAG GGC AAG ATT GTC CTG GAG GAC GGC ACA CTT CAT GTC ACT 1408

 E G S G R Y I P R K P F P D F V Y K 480
 GAA GGC TCA GGA CGC TAC ATT CCC CGG AAG CCC TTC CCT GAC TTT GTG TAC AAA 1462

 R I K A R S R L A E L R G V P R G L 498
 CGC ATC AAA GCA AGG AGC AGG CTG GCT GAG CTG AGA GGG GTC CCT CGT GGC CTG 1516

 Y D G P V C E V S V T P K T V T P A 516
 TAT GAC GGA CCG GTA TGC GAG GTG TCT GTG ACG CCC AAG ACG GTG ACT CCA GCC 1570

FIG.9 continued

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S S A K T S P A K Q Q Q A P P V R N L 534
 TCA TCA GCT AAG ACA TCC CCT GCC AAG CAG CAG CCA CCT GTT CGG AAC CTG 1624

H Q S G F S L S G A Q I D N I P R 552
 CAC CAG TCT GGA TTC AGC TTG TCT GGT GCT CAG ATT GAC GAC AAC ATT CCC CGC 1678

R T T Q R I V A P P G G R A N I T S 570
 CGC ACC ACC CAG CGC ATC GTG GCA CCC CCT GGT GGC GGT GCC AAC ATC ACC AGC 1732

L G * 573
 CTG GGC TAA agccctagccctgcagccacttggggatggggatgggacacctgaggacattctga 1800

gacttcctttcttccat

FIG. 9 continued

ULIP3 mouse

[illegible]

FIG. 10

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S	C	C	D	Y	S	L	H	V	D	I	T	S	W	Y	D	G	V	148
TCC	TGC	TGT	GAC	TAT	TCC	CTC	CAC	GTG	GAC	ATC	ACA	AGC	TGG	TAT	GAT	GGT	GTT	713
R	E	E	L	E	V	L	V	Q	D	K	G	V	N	S	F	Q	V	166
CGG	GAA	GAG	CTG	GAG	GTG	CTG	GTG	CAG	GAC	AAA	GGT	GTC	AAC	TCC	TTC	CAA	GTC	767
Y	M	A	Y	K	D	L	Y	Q	M	S	D	S	Q	L	Y	E	A	184
TAC	ATG	GCG	TAT	AAG	GAC	CTG	TAC	CAG	ATG	TCT	GAC	AGC	CAG	CTG	TAT	GAA	GCC	821
F	T	F	L	K	G	L	G	A	V	I	L	V	H	A	E	N	G	202
TTC	ACC	TTC	CTT	AAG	GGT	TTG	GGA	GCT	GTG	ATC	TTA	GTC	CAT	GCA	GAA	AAT	GGA	875
D	L	I	A	Q	E	Q	K	R	I	L	E	M	G	I	T	G	P	220
GAT	TTG	ATA	GCT	CAG	GAA	CAA	AAA	CGG	ATC	CTG	GAG	ATG	GGC	ATC	ACG	GGT	CCC	929
E	G	H	A	L	S	R	P	E	E	L	E	A	E	A	V	F	R	238
GAG	GGT	CAT	GCT	CTG	AGC	AGA	CCC	GAG	GAG	CTG	GAG	GCC	GAG	GCT	GTG	TTC	CGG	983
A	I	A	I	A	G	R	I	N	C	P	V	Y	I	T	K	V	M	256
GCT	ATT	GCC	ATT	GCA	GGC	CGG	ATC	AAT	TGC	CCT	GTG	TAC	ATC	ACC	AAG	GTC	ATG	1037
S	K	S	A	A	D	I	I	A	L	A	R	K	K	G	P	L	V	274
AGC	AAG	AGT	GCA	GCG	GAC	ATC	ATC	GCA	CTG	GCC	AGG	AAG	AAA	GGC	CCT	CTT	GTC	1091
F	G	E	P	I	A	A	S	L	G	T	D	G	T	H	Y	W	S	292
TTC	GGT	GAG	CCC	ATA	GCC	GCC	AGC	CTG	GGA	ACC	GAT	GGC	ACC	CAC	TAC	TGG	AGC	1145
K	N	W	A	K	A	A	A	F	V	T	S	P	P	L	S	P	D	310
AAG	AAC	TGG	GCC	AAG	GCA	GCT	GCA	TTT	GTG	ACT	TCC	CCT	CCC	CTG	AGC	CCA	GAC	1199

FIG.10 continued

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P	T	T	P	D	Y	L	T	S	L	L	A	C	G	D	L	Q	V	328
CCC	ACC	ACT	CCT	GAC	TAC	TTG	ACC	TCC	TTG	CTG	GCC	TGT	GGA	GAC	TTG	CAG	GTC	1253
T	G	S	G	H	C	P	Y	S	I	A	Q	K	A	V	G	K	D	346
ACA	GGT	AGT	GGC	CAC	TGT	CCC	TAC	AGT	ATT	GCT	CAG	AAG	GCT	GTG	GCC	AAG	GAC	1307
N	F	T	L	I	P	E	G	V	N	G	I	E	E	R	M	T	V	364
AAC	TTC	ACT	CTG	ATC	CCT	GAG	GGT	GTC	AAT	GGT	ATA	GAA	GAG	CGG	ATG	ACC	GTT	1361
V	W	D	K	A	V	A	T	G	K	M	D	E	N	Q	F	V	A	382
GTC	TGG	GAC	AAG	GCA	GTG	GCT	ACT	GGC	AAG	ATG	GAT	GAG	AAC	CAG	TTT	GTA	GCC	1415
V	T	S	T	N	A	A	K	I	F	N	L	Y	P	R	K	G	R	400
GTC	ACC	AGC	ACC	AAC	GCA	GCC	AAG	ATC	TTC	AAC	CTG	TAC	CCG	AGG	AAA	GGT	CGG	1469
I	A	V	G	S	D	A	D	V	V	I	W	D	P	D	K	M	K	418
ATC	GCT	GTG	GGC	TCC	GAT	GCT	GAC	GTA	GTC	ATC	TGG	GAC	CCA	GAT	AAG	ATG	AAG	1523
T	I	T	A	K	S	H	K	S	T	V	E	Y	N	I	F	E	G	436
ACC	ATA	ACA	GCC	AAA	AGC	CAT	AAA	TCA	ACT	GTG	GAG	TAC	AAC	ATC	TTT	GAG	GGC	1577
M	E	C	H	G	S	P	L	V	V	I	S	Q	G	K	I	V	F	454
ATG	GAG	TGC	CAC	GGC	TCC	CCC	CTG	GTG	GTC	ATC	AGT	CAG	GGC	AAG	ATT	GTC	TTT	1631
E	D	G	N	I	S	V	S	K	G	M	G	R	F	I	P	R	K	472
GAG	GAT	GGA	AAC	ATC	AGT	GTC	AGC	AAG	GGC	ATG	GGC	CGC	TTC	ATC	CCT	CGG	AAG	1685
P	F	P	E	H	L	Y	Q	R	V	R	I	R	S	K	V	F	G	490
CCA	TTC	CCA	GAG	CAT	CTC	TAC	CAG	CGT	GTC	AGG	ATC	AGA	AGC	AAG	GTT	TTC	GGG	1739

FIG.10 continued

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L H S V S R G M Y D G P V Y E V P A 508
 TTG CAT AGT GTT TCC AGG GGC ATG TAC GAT GGG CCT GTG TAC GAG GTG CCA GCT 1793

 T P K H A A P A P S A E S S P S K H 526
 ACA CCC AAA CAT GCT GCT CCT GCT TCT TCT GCC GAA TCC TCG CCT TCT AAA CAC 1847

 Q P P P I R N L H Q S N F S L S G A 544
 CAA CCC CCA CCC ATC CGG AAC CTC CAC CAG TCC AAC TTC AGC TTA TCA GGT GCC 1901

 Q I D D N N P R R T G H R I V A P P 562
 CAG ATA GAT GAC AAC AAT CCA AGG CGT ACA GGC CAC CGC ATT GTG GCG CCC CCT 1955

 G G R S N I T S L G * 573
 GGT GGC CGC TCC AAC ATC ACC AGC CTC GGT TGA cctcagatgagccagatatgcaagagt 2015

 gaaggattatgggaaaaacgtccattccttttccgtgtttttgaagccccacagtttttagttggtactgacgga 2087

 ggggagattgagcgatgctcttctctgttttaggaagaagtggtagttagtggtgtgttgccctgga 2159

 agtccctcgccacagtggtgtgttcacaccgactccacctcagagcatgggtgcggtccgttttcccttcta 2231

 gtgaccccggttagcatcgctcctatactgttccctccactcctccatgacccctctgagtgatgg 2297

FIG.10 continued

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ULIP4 mouse

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gctgactaatatgcttaaatcaggggtgccacgtctgtcggtacgtccacgccccgcgagccccctacc 72
gaggacactcagccccgcgtgtatcagg  ATG FCC TTC CAA GGC AAG AAG AGC ATT CCC 10
131
R I T S D R L L I K G G K I V N D D 28
CGG ATA ACG AGC GAC CGC CTT CTC ATC AAA GGT GGG AAG ATT GTG AAC GAT GAC 185
Q S F H A D L Y V E D G L I K Q I G 46
CAG TCC TTT CAT GCT GAT CTG TAT GTG GAA GAC GGT CTG ATT AAA CAA ATT GGA 239
E N L I V P G G I K T I D A H G L M 64
GAA AAT CTC ATC GTC CCT GGC GGC ATC AAA ACC ATC GAT GCT CAT GGC CTG ATG 293
V L P G G V D V H T R L Q M P V M G 82
GTG CTG CCT GGC GGA GTT GAC GTT CAC ACC CGG CTG CAG ATG CCT GTG ATG GGC 347
M T P A D D F C Q G T K A A L A G G 100
ATG ACC CCA GCT GAT GAT TTC TGT CAG GGC ACC AAG GCG GCT CTA GCA GGC GGG 401
T T M I L D H V F P D A G V S L L A 118
ACC ACC ATG ATA TTG GAC CAT GTG TTT CCT GAC GCT GGT GTG AGC CTG CTG GCA 455
A Y E Q W R D G A D S A A C C D Y S 136
GCC TAT GAG CAG TGG CGG GAC GGA GCA GAC AGC GCG GCC TGC TGT GAC TAC TCC 509
L H V D I P R W H E S T K E L E A 154
TTA CAT GTG GAC ATT CCT CGC TGG CAC GAG AGC ACC AAA GAA GAG CTG GAG GCC 563

```

FIG.11

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L V R D K G V N S F L V F M A Y K D	172
CTA GTC AGG GAC AAA GGT GTG AAC TCC TTC CTG GTC TTC ATG GCA TAC AAG GAC	617
R C Q C T D G Q I Y E I F S L I R D	190
AGG TGC CAG TGT ACT GAC GGT CAG ATA TAT GAA ATC TTC AGC CTC ATC CGG GAC	671
L G A V A Q V H A E N G D I V E E E	208
CTG GGA GCT GTG GCC CAG GTG CAC GCA GAA AAT GGG GAC ATC GTG GAG GAG GAA	725
Q K R L L E Q G I T G P E G H V L S	226
CAG AAG CGC CTG CTG GAG CAA GGC ATC ACT GGT CCT GAG GGC CAT GTG CTC AGC	779
H P E E V E A A E A V Y R A V T I A K	244
CAC CCA GAA GAG GTA GAG GCC GAG GCT GTG TAC AGA GCA GTC ACC ATT GCC AAG	833
Q A N C P L Y Y V T K V M S K G A A D	262
CAG GCC AAC TGC CCA CTA TAC TAC GTC ACC AAG GTG ATG AGC AAG GGT GCA GCT GAC	887
M V A Q A K R R G V V V F G E P I T	280
ATG GTT GCC CAA GCC AAG CGC AGG GGG GTG GTC GTC TTT GGG GAA CCT ATC ACT	941
A S L G T D G S H Y W S K N W A K A	298
GCC AGC CTG GGC ACT GAT GGC TCA CAC TAC TGG AGC AAG AAC TGG GCC AAG GCT	995
A A F V T S P P I N P D P T A D H	316
GCA GCC TTT GTC ACT TCA CCC CCT ATC AAC CCG GAC CCT ACT GCA GAC CAC	1049
L T S L L S S G D L Q V T G S A H C	334
CTC ACC TCT CTG CTG TCC AGT GGG GAC CTC CAG GTG ACA GGC AGT GCC CAC TGC	1103

FIG.11 continued

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T F T T A Q K A V G K D N F T L I P 352
ACC TTC ACT ACT GCC CAG AAG GCT GTT GGC AAA GAC AAC TTC ACA CTG ATC CCC 1157

E V V N G I E E R M S V V W E K C V 370
GAG GTA GTC AAC GGT ATA GAA GAG CGC ATG TCT GTG GTC TGG GAG AAA TGT GTG 1211

A S G K M D E N E F V A V T S T N A 388
GCT TCA GGG AAA ATG GAC GAG AAT GAG TTC GCT GCC GTG ACC AGC ACA AAT GCT 1265

A K I F N F Y P R K G R V A V G S D 406
GCC AAA ATC TTC AAT TTT TAC CCC AGG AAG GGG CGT GTG GCC GTG GGC TCT GAT 1319

A D L V I W N P R A T K V I S A K S 424
GCT GAC CTG GTC ATC TGG AAC CCC AGG GCC ACG AAA GTC ATC TCT GCC AAG AGC 1373

H N L N V E Y N I F E G V E C R G V 442
CAT AAC CTG AAT GTA GAG TAC AAC ATC TTT GAA GGA GTG GAG TGC CGA GGA GTG 1427

P T V V I S Q G R V V L E D G N L L 460
CCC ACG GTG GTC ATA AGT CAG GGC AGA GTG CTG GTG GAC GGA AAC CTG CTT 1481

V T P G A G R F I P R K T F P D F V 478
GTC ACT CCA GGG GCT GGC CGC TTC ATT CCC CGG AAG ACC TTC CCG GAC TTT GTG 1535

Y K R I K A R N R L A E I H G V P R 496
TAT AAG AGG ATA AAG GCT CGC AAC AGG CTA GCA GAG ATC CAC GGT GTG CCT CGA 1589

FIG.11 continued

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[illegible]

FIG. 11 continued

OLIP4 man

[illegible]

FIG. 12

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L V K E K G V N S F L V F M A Y K D 172
 CTG GTC AAG GAG AAG GGT GTG AAC TCC TTC CTG GTC TTC ATG GCA TAC AAG GAC 547
 R C Q C S D S Q M Y E I F S I I R D 190
 CGG TGC CAG TGC AGC GAC AGC CAG ATG TAC GAG ATC TTC AGC ATC ATC CGG GAC 601
 L G A L A Q V H A E N G D I V E E E 208
 CTG GGG GCC TTG GCC CAG GTG CAC GCT GAG AAC GGG GAC ATC GTG GAG GAG GAC 655
 Q K R L L E L G I T G P E G H V L S 226
 CAG AAG CGG TTG CTG GAG CTC GGC ATC ACT GGC CCC GAG GGC CAC GTG CTC AGC 709
 H P E V E A A E A V Y R A V T I A K 244
 CAC CCC GAG GAG GTG GAG GCT GAG GCG GTG TAC CGA GCT GTC ACC ATC GCC AAG 763
 Q A N C P L Y V T K V M S K G A A D 262
 CAG GCA AAC TGC CCG CTG TAC GTC ACC AAG GTG ATG AGC AAG GGG GCG GCC GAC 817
 A I A Q A K R R G V V V F G E P I T 280
 GCC ATC GCT CAG GCC AAG CGC AGA GGG GTG GTC GTG TTT GGG GAG CCC ATC ACC 871
 A S L G T D G S H Y W S K N W A K A 298
 GCC AGC CTG GGC ACC GAC GGT TCA CAC TAC TGG AGC AAG AAC TGG GCG AAG GCT 925
 A A F V T S P P V N P D P T A D H 316
 GCA GCC TTC GTC ACA TCA CCC CCT GTC AAC CCA GAC CCC ACC ACG GCA GAC CAC 979
 L T C L L S S G D L Q V T G S A H C 334
 CTC ACC TGC TTG CTG TCC AGC GGG GAC CTC CAG GTG ACA GGC AGC GCC CAC TGC 1033

FIG.12 *continued*

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T F T T A Q K A V G K D N F A L I P 352
 ACC TTC ACC ACT GCC CAG AAG GCT GTG GGC AAG GAC AAC TTC GCG CTG ATC CCC 1087

 E G T N G I E E R M S M V W E K C V 370
 GAG GGC ACC AAC GGC ATT GAG GAG CGC ATG TCG ATG GTC TGG GAG AAA TGT GTG 1141

 A S G K M D E E N E F V A V T S T N A 388
 GCC TCT GGG AAG ATG GAC GAG AAT GAG TTC GTC GCG GTG ACC AGT ACA AAT GCT 1195

 A K I F N F Y P R K G R V A V G S D 406
 GCC AAT ATC TTC AAT TTT TAC CCA AGG AAG GGG CGA GTG GCT GTG GGC TCT GAC 1249

 A D L V I W N P K A T K I I S A K T 424
 GCT GAC CTG GTC ATA TGG AAC CCC AAG GCC ACC AAG ATC ATC TCT GCC AAG ACC 1303

 H N L N V E Y N I F E G V E C R G A 442
 CAC AAT CTG AAC GTG GAG TAC AAC ATC TTC GAG GGA GTG GAG TGC CGG GGA GCG 1357

 P A V V I S Q G R V A L E D G K M F 460
 CCT GCC GTG GTC ATA AGT CAG GGC CGA GTG GCG CTG GAG GAC GGC AAG ATG TTT 1411

 V T P G A G R F V P R K T F P D F V 478
 GTC ACC CCG GGG GCG GGC CGC TTC GTC CCT CGG AAA ACA TTC CCG GAC TTT GTC 1465

 Y K R I K A R N R L A E I H G V P R 496
 TAC AAG AGG ATC AAA GCT CGC AAC AGG CTG GCG GAG ATC CAC GGT GTG CCC CGT 1519

FIG.12 continued

FIG.12 continued

TOTAL P.25

DECLARATION FOR PATENT APPLICATION AND APPOINTMENT OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention (Design, if applicable) entitled "Utilisation des protéines ULIP dans le diagnostic et la
thérapie des cancers et des syndromes neurologiques paranéoplasiques".

the specification of which (check one):

- ☐ is attached hereto.
- ☒ was filed on 17/08/99 as Application Serial No. 367,496,
 and was amended on _____ (if applicable).
- ☒ was filed on 19/02/98 as International Application (PCT) No. PCT/FR98/00328,
 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with *Title 37, Code of Federal Regulations, §1.56(a)*. I hereby claim foreign priority benefits under *Title 35, United States Code §119* of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

PRIOR FOREIGN APPLICATION(S)			PRIORITY CLAIMED	
<u>97 01 961</u>	<u>FRANCE</u>	<u>February 19, 1997</u>	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
Number	Country	Day/Month/Year Filed		
Number	Country	Day/Month/Year Filed	<input type="checkbox"/> Yes	<input type="checkbox"/> No
Number	Country	Day/Month/Year Filed	<input type="checkbox"/> Yes	<input type="checkbox"/> No
Number	Country	Day/Month/Year Filed	<input type="checkbox"/> Yes	<input type="checkbox"/> No

I hereby claim the benefit under *Title 35, United States Code, §120* of any United States application(s) or PCT international application(s) designating The United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of *Title 35, United States Code, §112*, I acknowledge the duty to disclose material information as defined in *Title 37, Code of Federal Regulations, §1.56(a)* which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

Application Number	Filing Date	Status - Patented, Pending or Abandoned

Application Number	Filing Date	Status - Patented, Pending or Abandoned

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under *section 1001 of title 18 of the United States Code* and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: I (We) hereby appoint as my (our) attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Andrew E. Taylor, Reg 17000, Thomas P. Sarro 19396-Harold L. Novick 26011-Mark J. Gutttag 33057, Walter C. Gillis 22086, Ross F. Hunt Jr 25082, Douglas E. Jackson 28518, Daniel C. Mallery 33532, Marvin Petry 22752, William E. Jacke 24016, B. Aaron Schulman 31877

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1-00

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State or Country	State or Country
Zip	Zip
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